The Thorax of Odonata (Insecta)

- including remarks on evolution and phylogeny

Dissertation

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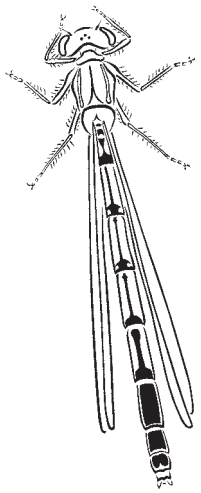

In Strichen, wo auf trocknem Land
Man Jungfraun nur noch selten fand
Sind Wasserjungfern, Demoisellen,
Libellen häufig festzustellen.
So kann der Mensch sich manchmal irren,
Sie scheinen reizend, wenn sie schwirren
Am Ufer hin, in Schilf und Gräsern
Mit ihren Flügeln, schön und gläsern.

Doch hat es jedem noch gegraut,
Der ihnen ins Gesicht geschaut:
Glotzaugen, bös, voll Mordverlangen
Und Kiefer, scharf wie Eisenzangen!

Eugen Roth

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1. General Introduction

1.1. Insecta

Insects are by far the richest in species and show an extraordinary variety of forms among living organisms (Figure 1). There are at least one million recent species described and this number increases annually by about 3000 species (Xylander & Günther 2003, Grimaldi & Engel 2005); estimates indicate the planet harbours 10 to 90 million insect species (Groombridge 1992, Wilson 1995). Many insects live together in enormous swarms and communities, e.g. locusts that occur in swarms ranging in size from 0.7 million to two million individuals (Groll & Günther 2003) or social hymenoptera, the colonies of which can grow to as many as 20 million individuals. The colony of the honeybee (*Apis mellifera* Linnaeus, 1758) can be made up of as many as 40 thousand to 100 thousand individuals (Dathe 2003a). There are therefore an estimated 10 sextillion (10^{21}) insect specimens currently living on Earth. That implies that there are around two billion insect individuals per human being (Dathe 2003b).

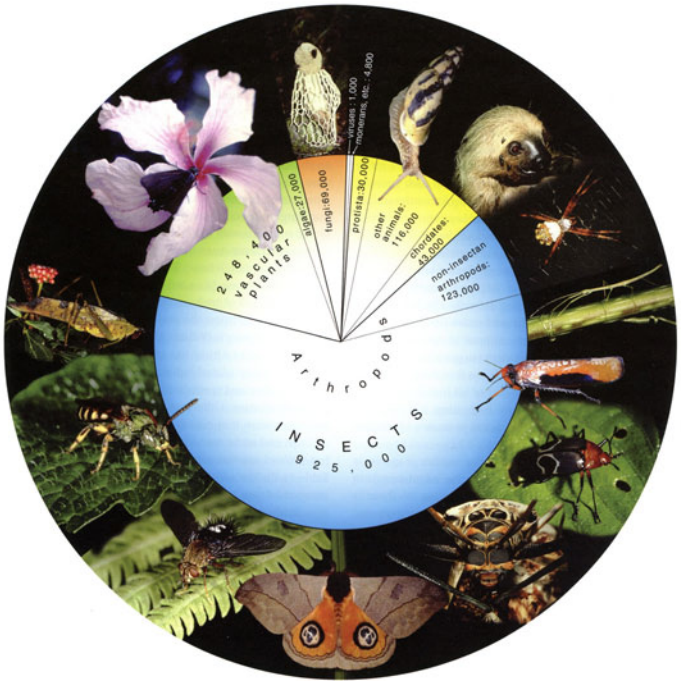

Figure 1 - The diversity of life shown as proportions of named species (Grimaldi & Engel 2005).

Insects are indisputably the most successful group of organisms alive. They are able to adapt to nearly all conceivable living conditions and habitats. Insects remained among the first life forms to conquer land for approximately 400 million years (MY). They may have arisen about 420 million years ago (MYA) in the Late Silurian. At this time, only a few other terrestrial organisms had colonized our world, with the most likely of them being other arthropods as well as plant species (Grimaldi & Engel 2005). In the case of insects, the key to their success and dispersability was the emergence of wings. Insects developed wings at least 90 MY earlier than vertebrates (Engel & Grimaldi 2004) and are the only invertebrates that have wings (Grimaldi & Engel 2005). Recently, the allegedly oldest pterygote insect, † *Strudiella devonica* (Garrouste, 2012), was discovered and interpreted as a winged devonian insect nymph (Garrouste et al. 2012). However, Hörnschemeyer et al. (2013) disproved this study and showed that † *S. devonica* was only a poorly preserved Devonian arthropod. The oldest known accepted winged fossil is † *Delitzschala bitterfeldensis* Brauckmann & Schneider, 1996.

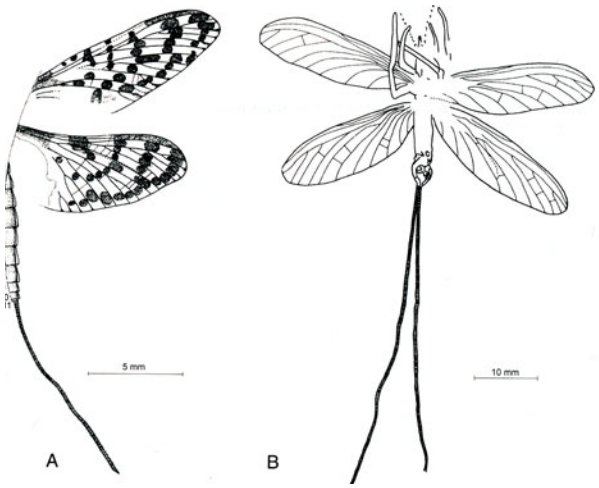

Figure 2 - Two of the oldest known pterygote insects. **A** † *Delitzschala bitterfeldensis* **B** † *Brodioptera stricklani*. (After Bruckmann & Schneider 1996 and Nelson & Tidwell 1988).

Dating back to the Lower Carboniferous, or approximately 320 MYA, it was found in Germany (Figure 2; Willmann 2003). Wings nevertheless remain the key to insect evolution and success. They are an indispensable character system that was 'perfected' in the Odonata.

1.2. Odonata

Odonata are one of the oldest members of this most diverse group of living organisms. This insect group (Figure 3), which includes 5500 recent and approximately 600 fossil species, is distributed worldwide with exception of the circumpolar regions. The greatest diversity is found in the Oriental Region with 1000 species, followed by the Neotropic and Ethiopic ecozones. There are 114 known species for Europe, with 80 known to be found in Central Europe (Xylander & Günther 2003, Grimaldi & Engel 2005).

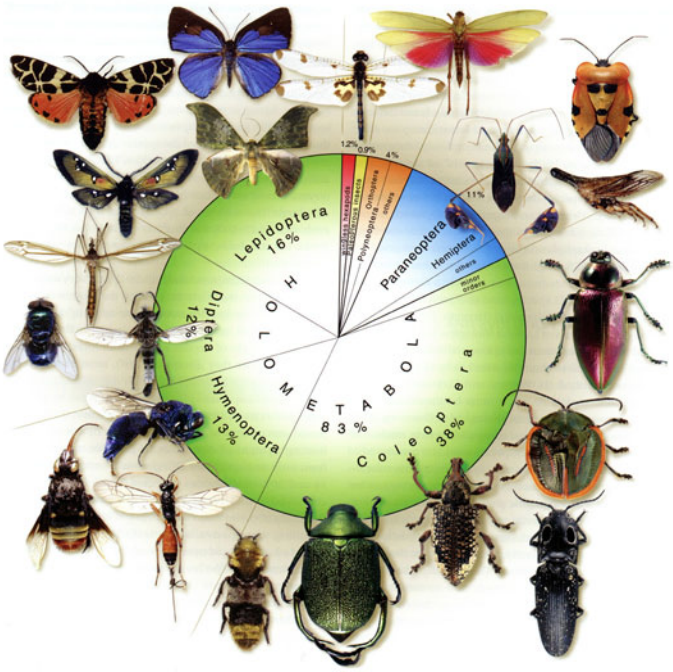

Figure 3 - The diversity of recent Insecta as proportions of named species (Grimaldi & Engel 2005).

Dragonflies and damselflies are undeniably one of the key arthropod predators. This applies to both nymphs, who live under the water surface and are able to hunt even for vertebrates like small fishes, and adults, which are skilled avian predators (e.g. Corbet 1999). Their wing pairs can be controlled independently and some species are able to even fly backwards (Hatch 1966). The variation between nymph and adult Odonata habitat – nymphs live under water and adults are terrestrial – bears comparison with

the Holometabola (e.g. Corbet 1999). Nymph and adult hemimetabolous insects generally share a common habitat (e.g. Deckert & Göllner-Scheiding 2003).

This superordinate position, a major role in the invertebrate food web, and adaption to totally different habitats of nymphs and adults, as mentioned above, are reflected in a unique morphology. The basic pattern morphology is comparably uniform (Figure 4); some of the most important characters of Odonata are:

Adults:

- Four long, uniform wings with distal pterostigma and highly derived wing venation.
- The prothorax is small and extremely versatile. The mesothorax and metathorax form a functional unit known as the pterothorax or synthorax, which is tilted caudally at 45° in Odonata.
- The pleurites are rather enlarged in a dorso-ventral direction, whereas the tergites and sternites are unusually small compared to other pterygotes.
- Direct muscle attachment is the mechanism for moving the wing. The dorsal longitudinal thorax musculature is reduced.
- Spined legs thrust forward for catching prey.
- The abdomen is slender, and can approaches a cigar shape. Secondary male genitalia are developed at the second and third sternite.
- The mouthparts are chewing and well-developed. This character is eponymous for this group. Derived from the Greek „odonto“ – meaning tooth, refers to the strong teeth found on the mandibles of most adults.

Nymphs:

- Highly specialized labium, called the prehensile mask.
- Respiration via gills, either within the hindgut (Epiprocta) or with three flabelliform appendices at the end of the abdomen (Zygoptera).
- Thorax uniform with developing wing buds

(Hennig 1959, Xylander & Günther 2003, Büsse et al. 2013, Genet et al. subm. Büsse & Hörnschemeyer subm.).

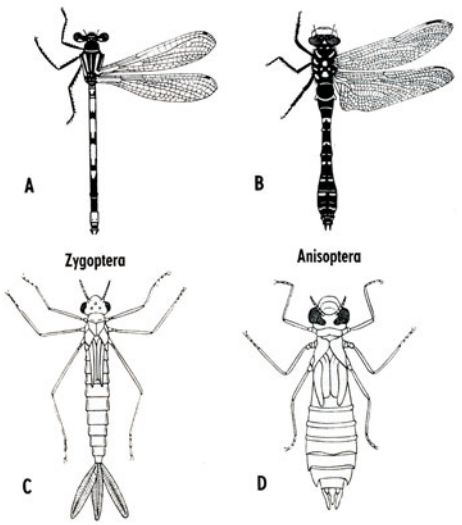

Figure 4 - Odonata
A,**C** Zygoptera. **B**,**D** Anisoptera.
A *Coenagrion puella* L., 1758. **B** *Cordulegaster boltonii* Donovan, 1807. **C** Nymph of *Lestes spec.*. **D** Nymph of *Anax imperator* Leach, 1815. (After Hennig 1959).

Generally, Odonata can be divided into three subordinate taxa: Two well-known groups, dragonflies (Anisoptera) and damselflies (Zygoptera) and a not commonly known and enigmatic group *Epiophlebia* (e.g. Bybee et al. 2008). The body size of adult Odonata varies considerably. The largest living species is *Megaloprepus caerulatus* (Drury, 1782) a Anisoptera with a body length of up to 150mm and a maximum wingspan of 200mm. Whereas the smallest recent Zygoptera, *Agriocnemis* Selys, 1877, and *Ischnura* Charpentier, 1840, have a body length of between 15 mm and 16 mm and a wingspan of around 20mm (Xylander & Günther 2003, Grimaldi & Engel 2005).

Extant species of *Epiophlebia* were historically grouped together with fossil taxa, forming the "Anisozygoptera" (e.g. Nel 1993). This name reflects the conspicuous mixture of anisopteran and zygopteran characters found in its extant species (Asahina 1954, Rüppell & Hilfert 1993, Xylander & Günther 2003, Büsse et al. 2012). The term is avoided in this work because Nel (1993) has already shown that the "Anisozygoptera" are a paraphyletic assemblage.

Epiophlebia's conspicuous mixture of zygopteran and anisopteran characters (Asahina 1954, Büsse et al. 2012) reflects the most ancestral character distribution of all known Odonata (e.g. Blanke et al. 2013a), meaning it occupies a special position.

1.3. Thorax Morphology

The insect thorax is the tagma of locomotion – walking in apterygote insects and additionally flying in pterygote insects. The thorax has three segments (prothorax, mesothorax and metathorax), comprises three pairs of legs and, in case of the pterygote basic pattern (e.g. Snodgrass 1935), two pairs of wings. The prothorax follows the head. This segment is connected to the thorax by the cervix, which is largely made up of membranous parts (neck; Snodgrass 1935). The mesothorax and metathorax comprise the entire flight apparatus in the Pterygota including all flight muscles. Therefore, this tagma can be called pterothorax or synthorax (Matsuda 1970, Chapman 1998).

The odonatan thorax is, however, a highly specialized and therefore a much derived character system. Therefore, a description of the more detailed characters of the insect thorax may be beyond the scope of this thesis (cf. Snodgrass 1935, Matsuda 1970).

The thorax of adult Odonata is made up of the small prothorax and pterothorax, which is caudally tilted at 45° (Figure 5). This is caused by the pleura, which constitutes the connection between the tergal and sternal regions. The strong ventral

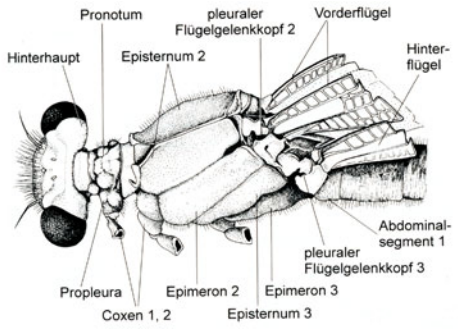

Figure 5 - *Calopteryx splendens* (Harris, 1782). The mesothorax and metathorax (pterothorax) is 45° caudally tilted. (After Hennig 1959).

and dorsal expansion of the pleura causes the convex elevation of the wing articulation in Odonata. The wing is connected by a membrane with the thorax to allow movability (Genet et al. subm.). Direct muscle attachment is the primary agent for this movability (Büsse et al. 2013).

The thorax morphology of adult Odonata is elaborately described and discussed by Büsse et al. (2013) and Genet et al. (subm.) and for nymphs by Büsse & Hörnschemeyer (subm.).

1.4 Phylogeny of Odonata

The sister group relationship of Odonata within the Insecta is controversial. The recently favoured hypothesis is the Palaeoptera hypothesis (e.g. Blanke et al. 2012a, 2012b). Martynov (1924) had already divided the Pterygota into two well-defined groups. He called them Palaeoptera – or "old winged" insects and Neoptera – or "new winged" insects. This hypothesis and two others on the relationship of Odonata, Ephemeroptera and Neoptera can be viewed in greater detail. The three subordinated taxa of Pterygota – Odonata, Ephemeroptera and Neoptera, which comprise all other insects, are grouped in all conceivable possibilities (Figure 6).

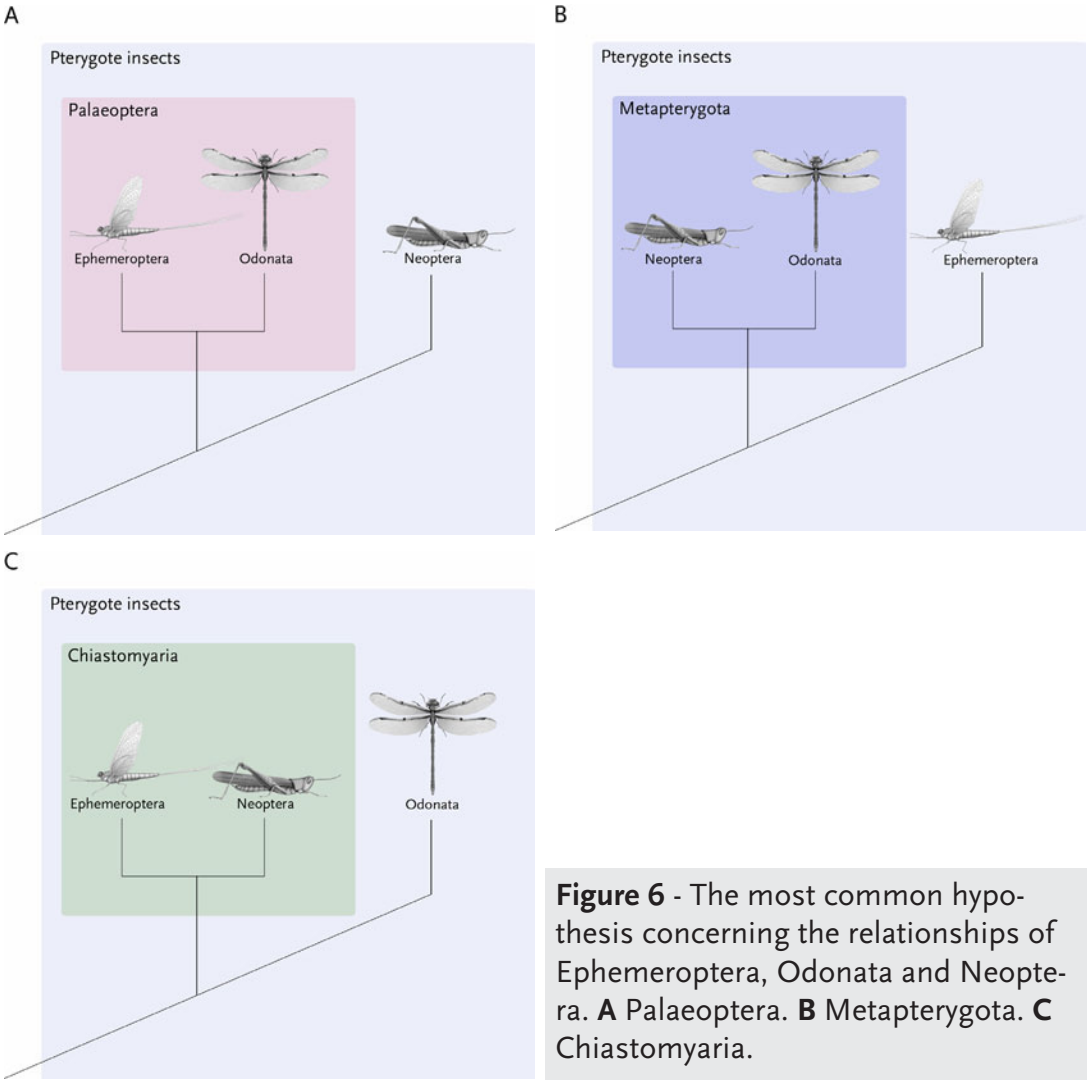

Figure 6 - The most common hypothesis concerning the relationships of Ephemeroptera, Odonata and Neoptera. **A** Palaeoptera. **B** Metapterygota. **C** Chiastomyaria.

The first hypothesis, mentioned above, is the Palaeoptera hypothesis. This hypothesis favours a sister group relationship of Ephemeroptera and Odonata and is reinforced by the most recent investigations of Blanke et al. (2012a, 2012b) and Thomas et al. (2013). Morphological evidence – for example the inability to fold their wings above the abdomen and the related similarity of wing base structures (Martynov 1924, Hennig 1969, Kukalová-Peck 1978, 1983, 1985, 1991, 2008, Wootton 1979, Bechly 1996, Haas & Kukalová-Peck 2001,Rasnitsyn 2002) – or DNA analysis (Hovmöller et al. 2002, Ishiwata et al. 2011) also supports this hypothesis.

The second hypothesis – the Metapterygota hypothesis – assumes a sister group relationship of Odonata and Neoptera. Shared characters, such as the lack of the ecdysis in the winged stage, the number and position of the mandible-articulations and associated loss of several muscles, etc. (Börner 1909, Hennig 1953, Kristensen 1975, 1981, 1991, Staniczek 2000, 2001, Wheeler et al. 2001, Willmann 2003, Grimaldi & Engel 2005, Beutel & Gorb 2006, Willkommen & Hörnschemeyer 2007), as well as a DNA analysis from Ogden and Whiting (2003) are used to support this hypothesis.

The third hypothesis is the Chiastomyaria hypothesis, representing the sister group relationship of Ephemeroptera and Neoptera (Bourdreaux 1979, Carle 1982a, 1982b). The strong dorso-longitudinal indirect wing depressor, often considered symplesiomorphic, and direct sperm transfer by the male to the female gonoporus, which was often considered as convergent, are characters for this hypothesis which has the least evidence supporting it (Willmann 2003).

Deeper understanding and a most widely accepted hypothesis is however indispensable for elucidating the ground pattern of ancestral Pterygota and understanding the origin of insect flight (Kingsolver & Koehl 1994). The controversy surrounding these hypotheses reflects a phenomenon observed in the early Pterygota; the disproportionately rapid divergence of the three lineages from a common ancestor. This accelerated radiation in pterygote evolution is underscored by a complete lack of stem group representatives and/or transitional forms in the fossil record. What is more, we know well-defined stem group members of all three lineages of Pterygota from the lowermost Upper Carboniferous (Carpenter 1992, Rasnitsyn & Quicke 2002, Grimaldi & Engel 2005).

The first group named in the context of odonatan phylogeny is primarily the extinct Protodonata or "griffenflies", called giant dragonflies (Figure 7).

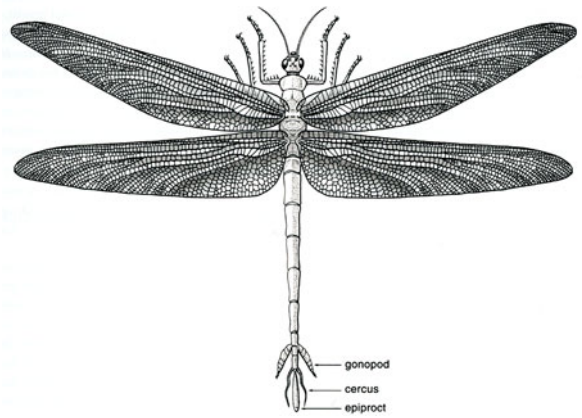

Figure 7 - Reconstruction of one of the largest, extinct odonatopterans, † *Namurotypus sippeli* Bechly, 2001, from the Carboniferous. (After Grimaldi & Engel 2005).

Figure 8 - Wing of † *Megatypus schucherti* (Meganeuridae: Protodonata) from the Early Permian. (After Grimaldi & Engel 2005).

However, the Protodonata might be the stem group of the Zygoptera and Epiprocta [Anisoptera+*Epiophlebia*]. Moreover, the largest insect that has ever lived belongs to that group: † *Meganeuropsis permiana* Carpenter, 1939, has a body length of up to 370mm and a wingspan of 750mm. The Protodonata from the Permian were comparatively similar to recent Odonata. This is mainly based on preserved wings (e.g. Figure 8) but the few preserved body parts described show large, toothed mandibles as well as enormous compound eyes and legs reflecting the typical odonatan position for catching prey (Grimaldi & Engel 2005).

Tarsophlebioptera (Figure 9) dating back to the Jurassic can be viewed as the sister group of all recent Odonata. For example † *Turanophlebia*, a member of Tarsophlebioptera (Figure 10), were primitive Odonata with characteristics of Epiprocta as well as Zygoptera (Rehn 2003, Grimaldi & Engel 2005). Since the beginning of the Miocene we find species that are relatively similar to recent Odonata (Figure 11; Grimaldi & Engel 2005).

Recent phylogenetic studies of the three main groups of Odonata based on morphological as well as molecular studies support the sister group relationship of Zygoptera and Epiprocta (Carle 1982, Bechly 1996, Lohmann 1996, Trueman 1996, Misof et al. 2001, Rehn 2003, Bybee et al. 2008, Gade et al. 2011). In only a single study is *Epiophlebia* designated the sister group of the Cordulegastridae (Anisoptera; Dumont et al. 2010). All other subgroup relationships are controversial and have yet to be completely addressed. For the sake of completeness, a cladogram showing one possible relationship within the Odonata (Figure 12) is presented (Bybee et al. 2008), but will not be discussed further.

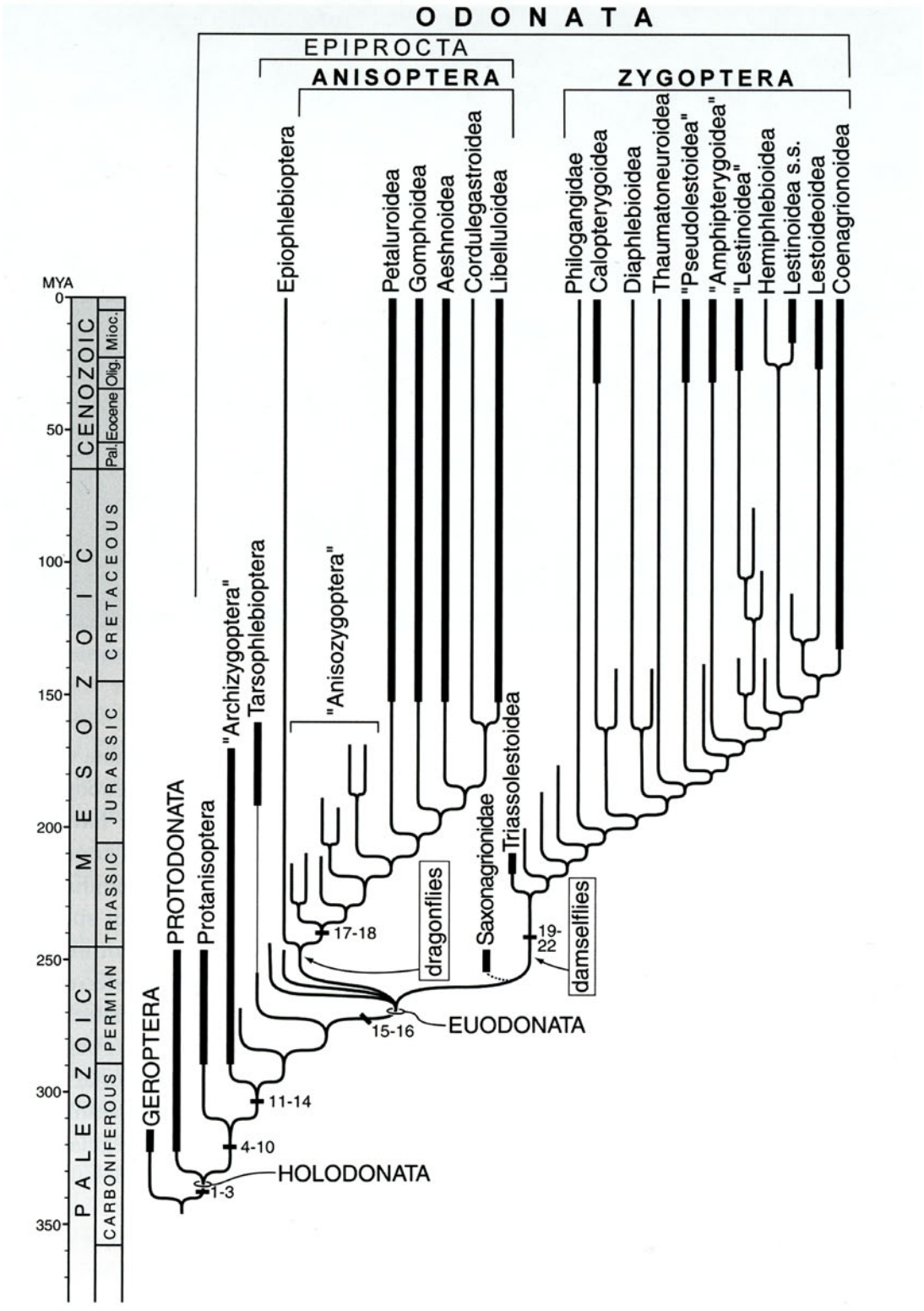

Figure 9 - Phylogeny of Odonatoptera (living odonates and their extinct relatives). (After Grimaldi & Engel 2005).

Figure 10 - A primitive odonate, † *Turanophlebia*, a member of Tarsophlebioptera from the Jurassic. (After Grimaldi & Engel 2005).

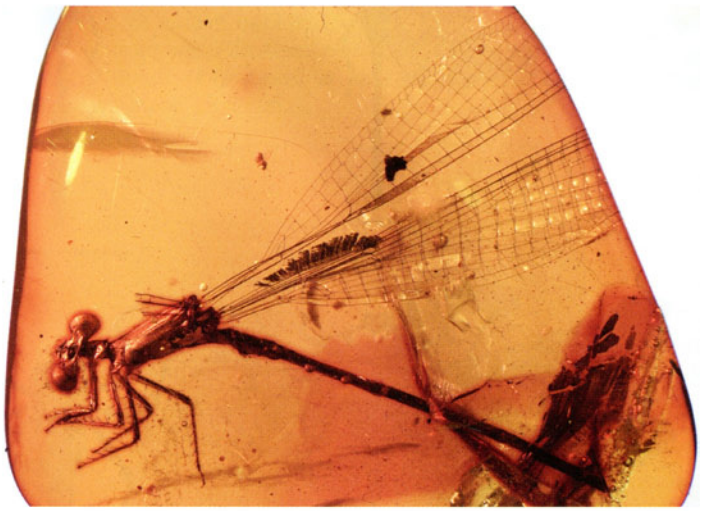

Figure 11 - Relatively modern Zygoptera in Miocene amber. (After Grimaldi & Engel 2005).

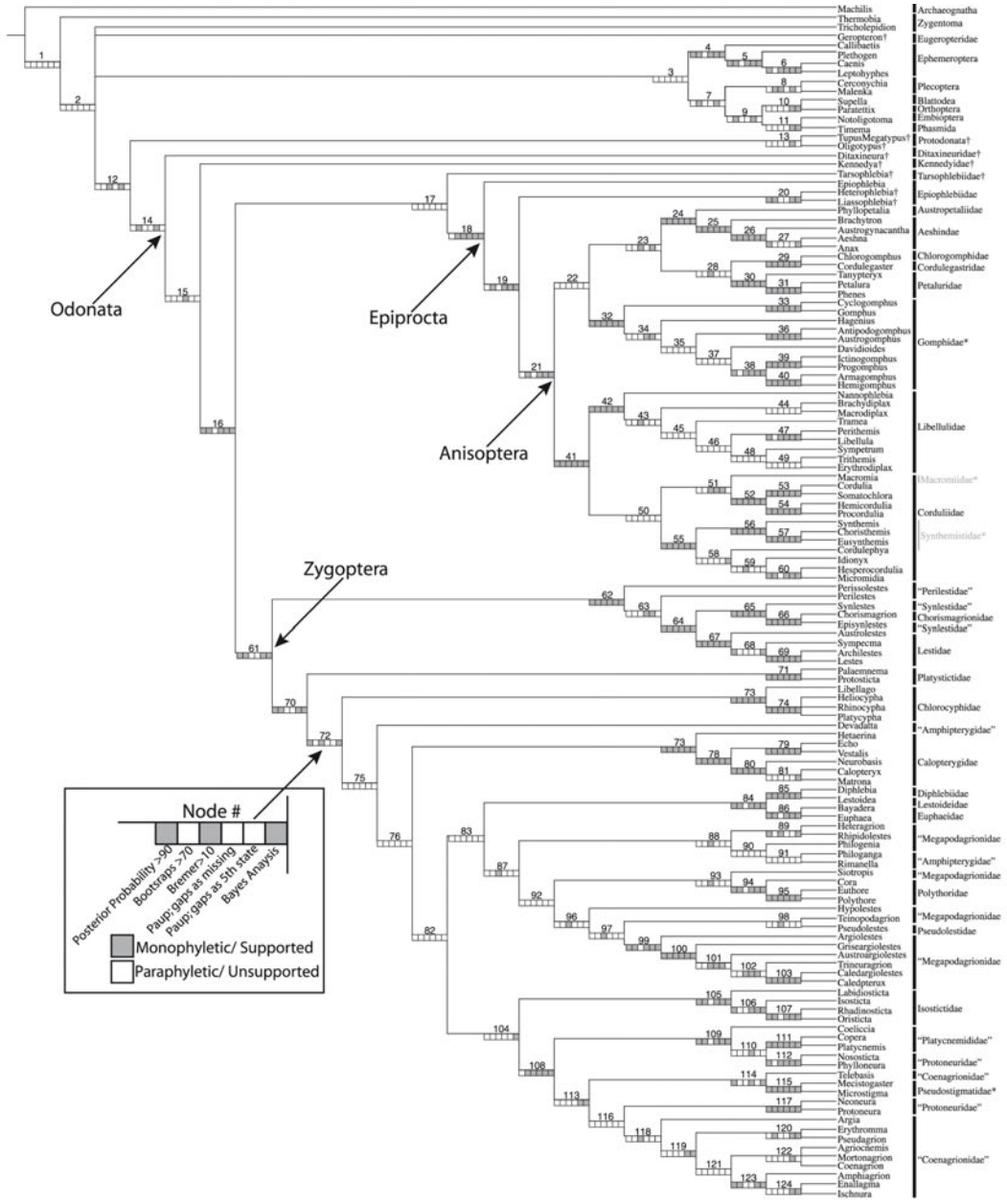

Figure 12 - Hypothetical phylogeny of Odonata. After Bybee et al. 2008).

1.5. Aims of the Present Study

As mentioned in the previous paragraphs, the phylogeny of Odonata and the homology of the odonatan flight apparatus with the neopteran counterparts have been only sparsely investigated and understood to date. The present study therefore aims to elucidate the morphology of the flight apparatus of Odonata and address some fundamental phylogenetic questions. The methodology used to achieve this consisted of DNA analysis, synchrotron radiation micro-computed tomography (SRμCT) aided by 3D-reconstruction, ESEM, stacked photography and light microscopy.

As part of the genetic investigations of the basal splits of Odonata, the sister group relationship of the relict dragonflies of *Epiophlebia* – *Epiophlebia superstes* (Sélys, 1889), *Epiophlebia laidlawi* Tillyard, 1921 and *Epiophlebia sinensis* Li and Nel, 2011 was studied (Büsse et al. 2012). The morphology of *Epiophlebia laidlawi* was additionally studied in more detail, to underscore the species status (Büsse in prep.).

To create a profound hypothesis of the homology of the thorax musculature (Büsse et al. 2013, Büsse & Hörnschemeyer subm.) and the wing base structures (Genet et al. subm.) of Odonata and Neoptera, a set of adult and nymphal Odonata were investigated. The data obtained are compared to literature as a contribution to elucidate the evolution of the odonatan thorax.

A more or less chance finding resulting from the genetic analysis of the badly preserved *Epiophlebia* species is a universal analysis system for taxonomic identification of Insecta species applicable for degraded DNA. This genetic tool is one of the simplest ways of identifying insect species, no matter what the specimen condition (e.g. Grumbkow et al. subm.).

2. Phylogeographic Analysis Elucidates the Influence of the Ice Ages on the Disjunct Distribution of Relict Dragonflies in Asia

2.1. Contribution to this Publication

Conceived and designed the experiments: SB PvG SH KY TH.
Performed the experiments: SB PvG TH.
Analyzed the data: SB PvG TH.
Contributed reagents/materials/analysis tools: PvG DNS RDTS JL XZ KY.
Wrote the paper: SB PvG TH SW.

2.2. Publication

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Phylogeographic Analysis Elucidates the Influence of the Ice Ages on the Disjunct Distribution of Relict Dragonflies in Asia

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Abstract

Unusual biogeographic patterns of closely related groups reflect events in the past, and molecular analyses can help to elucidate these events. While ample research on the origin of disjunct distributions of different organism groups in the Western Palearctic has been conducted, such studies are rare for Eastern Palearctic organisms. In this paper we present a phylogeographic analysis of the disjunct distribution pattern of the extant species of the strongly cool-adapted *Epiophlebia* dragonflies from Asia. We investigated sequences of the usually more conserved 18 S rDNA and 28 S rDNA genes and the more variable sequences of ITS1, ITS2 and CO2 of all three currently recognised *Epiophlebia* species and of a sample of other odonatan species. In all genes investigated the degrees of similarity between species of *Epiophlebia* are very high and resemble those otherwise found between different populations of the same species in Odonata. This indicates that substantial gene transfer between these populations occurred in the comparatively recent past. Our analyses imply a wide distribution of the ancestor of extant *Epiophlebia* in Southeast Asia during the last ice age, when suitable habitats were more common. During the following warming phase, its range contracted, resulting in the current disjunct distribution. Given the strong sensitivity of these species to climatic parameters, the current trend to increasing global temperatures will further reduce acceptable habitats and seriously threaten the existences of these last representatives of an ancient group of Odonata.

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Introduction

Disjunct biogeographic patterns with closely related organisms occurring in widely separated areas are puzzling. Such disjunct distributions reflect historical events, and a major goal of research is to deduce which events were responsible for the given distribution pattern. Disjunct ranges of closely related taxa can come into existence either by tectonics, by dispersal or by intervening extinction [1]. For European mountain ranges it has already been shown that today's disjunct distribution patterns of many cold-adapted species are post ice age relicts [2]. However, the study of disjunct species and their phylogeography in other areas such as e.g. the Himalayas and Southeast Asia is still in its infancy.

In this paper we illuminate the disjunct distribution pattern of *Epiophlebia*, a unique dragonfly from Asia.

Traditionally, the Odonata have been divided into Anisoptera (dragonflies), Zygoptera (damselflies) and "Anisozygoptera". Besides the extant species of *Epiophlebia* the "Anisozygoptera" comprised mainly Jurassic fossils [3] until it was shown that "Anisozygoptera" are not monophyletic [3–5]. Presently, *Epiophlebia* is considered to be the most basal sistergroup of the Anisoptera, with several extinct lineages nested in between [4–7]. A close relationship of Anisoptera and *Epiophlebia* has also been corroborated by several molecular analyses and the term Epiprocta has been introduced for this grouping [8–11].

The species of *Epiophlebia* have been considered as "living fossils" [12] because they display features of both damselflies (Zygoptera) and dragonflies (Anisoptera). In their general body outline they resemble dragonflies, but as in damselflies their fore- and hind wings are similarly shaped and petiolate [12]. Like dragonflies, the larvae use a rectal chamber for respiration, but jet propulsion, which is typical of dragonflies, was never observed [13].

So far it was assumed that there are three extant species of *Epiophlebia* but there were doubts on the species differentiation between *E. superstes* (Sélys, 1889) and *E. laidlawi* Tillyard, 1921 from early on [12,14–17]. Recently, a third species *E. sinensis* Li and Nel, 2011 was described from China [18].

While the Japanese *E. superstes* was thoroughly described by Asahina [12,16], *E. laidlawi*, and *E. sinensis* are only poorly known. The morphological discrimination between species is based only on the following features [16,17,19]: adults of *E. laidlawi* differ from *E. superstes* in larger size and brownish body coloration, parts of the male genitalia differ slightly in shape, the apical process of the eighth sternite is less developed in the female of *E. laidlawi*, and the wings are slightly longer [17]. For the larvae of *E. laidlawi* the following differences to *E. superstes* have been descibed [16: p. 445]: slightly larger body size; length and form of the third antennal segment; form of the antero-lateral angle of the pronotum, form of the fore femur, development of the lateral spines of abdominal segments 7 to 9, and differences in the shape of the epiproct.

E. sinensis was described on the base of two adult male specimens, which differ from the other species in the hairiness of the epiproct and in the colouration of the abdomen [18].

Nowadays, the distribution of *Epiophlebia* is disjunct. *E. superstes* is restricted to large areas of Japan [12,13,20], *E. laidlawi* is found only in the Himalayas [14,16,19,21] and the recently described *E. sinensis* adds another dot in north-east China to the disjunct pattern. This distribution is due to very specific habitat requirements. *Epiophlebia* prefers cold mountain streams with temperatures of about 4 to 5°C in winter and about 16–17°C in summer (data published for *E. superstes* [13]) and altitudes between 1,300 to approximately 3,000 m (for *E. laidlawi* [21]). The recently described *E. sinensis* also fits into this pattern as it was collected in the vicinity of a mountain stream, however, at an elevation of not more than 500 m [18]. In a recent discussion the biogeography of *E. superstes* and *E. laidlawi* [21] Epiophlebiidae and the extinct closely related Stenophlebiidae are considered as part of an "archeo-palaearctic dragonfly fauna" that formed in the Mesozoic and during the Tertiary was intermingled with oriental faunal elements. The extant *Epiophlebia* is considered as belonging to this ancient fauna, parts of which survived on the Japanese islands, in the Himalayas and in China [18,21].

The present study aims at clarifying the phylogenetic relationships and the biogeographic history of *E. superstes*, *E. laidlawi* and *E. sinensis* from a genetic point of view. Since DNA sequences so far were only available for *E. superstes* [8,22,23] we had to acquire additional sequence data for specimens from the other species and from different populations. The specimens available had originally been preserved for morphological and faunistic research, applying preservatives containing, among others, formaldehyde. Thus, we applied techniques used for analysis of degraded DNA [24] to get sequences of sufficient length. To achieve a good resolution of relationships on all taxonomic levels we investigated conserved genes as well as more variable regions [8,25,26].

Here we present the unexpected homogeneity of DNA-sequences of the supposed species *E. laidlawi*, *E. superstes* and *E. sinensis*. In the light of our new data, the extant disjunct distribution of *Epiophlebia* is explained in a distinctly different scenario than those suggested by [21] or [18].

Results

The specimens of *Epiophlebia* initially were not collected and preserved for subsequent DNA analysis. Thus, amplification and sequencing turned out to be problematic. However, for all targeted genes it was possible to acquire sections of up to 300 bp (Table S1). These were positioned in such a way, that phylogenetic relevant sequences could be expected while allowing straightforward alignment throughout at least the Odonata.

For *E. superstes* and *E. laidlawi* sequences of 18 S, and 28 S rDNA, ITS1, ITS2 and CO2 genes were analysed as well as CO1 for *E. superstes*. For *E. laidlawi* it was not possible to get a sequence for CO1. Due to contamination with other odonatan DNA we could only acquire the ITS sequences for *E. sinensis*, because our primers are highly specific for these genes.

All sequences show an extreme degree of similarity between all *Epiophlebia* species.

DNA Analysis

Sequences of *E. superstes* from [8] were used as reference. The sequences of 18 S rRNA (240 bp) and 28 S rRNA genes (1:191 bp; 2:267 bp; 3:251 bp; 4:293 bp) did not show any differences between *E. laidlawi* (specimens NATR3 & NA01) and *E. superstes* (our sequences and FN356086, EU424328). For CO2 (265 bp) a single difference at position 368 relative to the *E. superstes* reference sequence (EU055421) was found in all specimens including the Japanese control specimen (Figure S1).

Likewise, the fragment of ITS2 (265 bp) shows one deletion of G at position 2613 (relative to reference sequence FN356086) in all specimens investigated. Additionally, a maximum of three more differences were found in one of the *E. laidlawi* specimens (NA01) the other specimen (NATR03) showed only one difference and in *E. sinensis* there are two (Figure S2).

In ITS1 (215 bp) two deletions (one G at position 1943 and one C at position 1944, relative to the *E. superstes* reference sequence from GenBank) are common to all specimens investigated. Additionally, ITS1 shows a maximum of 11 differences in *E. laidlawi* and seven in *E. sinensis*. In specimen NA01 of *E. laidlawi* also a heterozygous duplication of AAC at position 1935 of ITS1 was detected (Figure 1).

Phylogenetic Analysis

The complete dataset for phylogenetic analysis contained 17 taxa including a representative of Zygentoma as outgroup, two specimens of both *Epiophlebia laidlawi* and *E. superstes* and one specimen of *E. sinensis*. For each gene one sequence for *E. superstes* was taken from GenBank (Table S1). The 4799 characters were composed of 537 positions from the CO2 gene, 1700 positions from 18 S, 2157 positions from 28 S, 231 nucleotides from ITS2 and 173 nucleotides from ITS1. For some of the taxa in the matrix sequences from different species, or specimens of uncertain species determination, of a certain genus had to be combined into a chimeric sequence. This is true for Zygentoma, Calopterygidae, Lestidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae and Coenagrionidae (Table S1). Alignment of ITS sequences turned out to be problematic due to their high variability. Eventually, an alignment was accepted, produced with the online version of MAFFT [27] with standard parameters, except for the scoring matrix set to "20PAM/k = 2" and the offset value set to 0.1. Highly variable sections were removed from the dataset.

Since it was not possible to obtain a sequence of CO1 for *E. laidlawi* or *E. sinensis*, this gene was not used in the phylogenetic analysis.

Data were formatted as mixed dataset for analysis with MrBayes 3.1.2. For each data partition a model was selected with MrModeltest 2.3: for CO2, 18 S and 28 S GTR+I+G was used, for ITS2 F81+G and for ITS1 JC+G. A NEXUS file with alignments and all parameters used can be found in Dataset S1.

Bayesian analysis over four million generations produced the phylogram shown in Figure 2A.

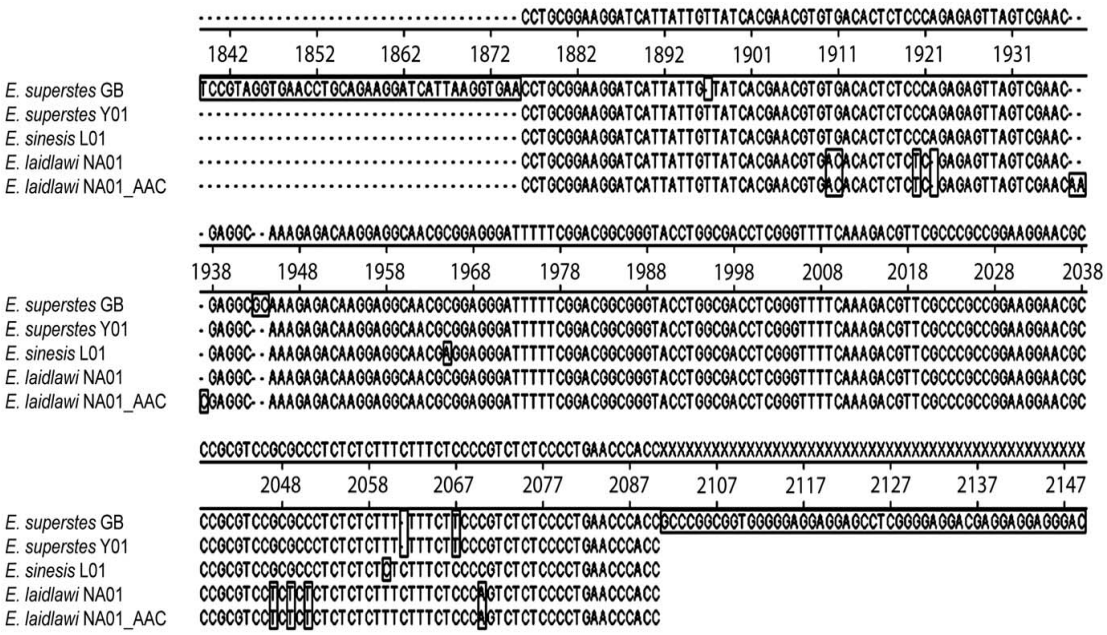

Figure 1. Alignment of ITS1 sequences from different specimens of *Epiophlebia* species. *E. superstes*_GB = reference sequence from GenBank.
doi:10.1371/journal.pone.0038132.g001

For the objective of this investigation the relationships of the *Epiophlebia* specimens are most important. These specimens do not appear in a sistergroup relationship with the other specimen of the same species. This result is reproduced when analysing the dataset with the maximum likelihood algorithm (ML) (SYM+I+G used for the complete dataset, Figure 2B) or under maximum parsimony (hsearch, addseq = random, nreps = 100; both in PAUP*). With respect to the relationships of the *Epiophlebia* specimens also phylogenetic analyses based only on single genes reproduced the same arrangement.

The position of *Epiophlebia* as sistergroup of Zygoptera and the non-monophyly of Anisoptera are remarkable. These results contradict the widely accepted hypothesis of a monophyletic Anisoptera and a recently published hypothesis based on more comprehensive datasets proposing a sistergroup relationship between *Epiophlebia* and Anisoptera [8–11]. However, in the maximum likelihood analysis (Figure 2B) a sistergroup relationship of *Epiophlebia* and a clade containing Anisoptera with the exception of Aeshnidae is recovered.

Morphology

The external morphology of several larval instars of *E. superstes* and *E. laidlawi* was compared by [16]. He concluded that they might well be two separate species based on six differing characters. Four of these characters could not be confirmed by our morphological investigation of larvae of both species. Two characters, the lateral posterior corners of the abdominal segments 7 to 9, and the shape of the epiproct consistently show slight differences between these supposed two species. Larvae of *E. sinensis* were not available for investigation.

Differences in the adults are restricted mainly to coloration. Himalayan specimens seem to be more brownish than the black Japanese specimens and the Chinese ones have a reddish hint in the posterior area of the abdomen. Furthermore, there are small differences in the arrangement of setae and in the shape of the male genitalia [12,14–18,28].

In total, the visible differences between specimens from the different regions allow identifying where a specimen was found. However, a general problem is the small number of known specimens from the Himalayas and from China. Thus the variability of morphological features cannot be determined.

Discussion

When assembling and aligning the sequences from *Epiophlebia superstes*, *E. laidlawi* and *E. sinensis*, it quickly became obvious that there are only very few differences between these supposed species.

In order to be able to adequately rate this observation, we compared sequences of several different closely related odonatan species as well as specimens of the same species from different populations (Table 1). Between representatives of different species there usually are significantly more differences over similar sequence length in the same area of a gene than are present between the specimens of *Epiophlebia*. In most species the number of intraspecific differences (e.g. in CO2 of *Ischnura asiatica*) is higher than the number of differences found between species of *Epiophlebia*. Even the usually variable partial 28 S sequences show no polymorphisms between the investigated specimens. We were only able to locate the more or less identical region of ITS from GenBank for other Odonata species. Unfortunately, for 18 S, 28 S and CO2 we were not able to locate the same sections in enough specimens of closely related species or of different specimens of the same species for such a comparison. However, due to the used sequencing method we identify the most variable regions of 18 S and 28 S and even those show no differences.

The occurrence of a heterogeneous insertion of three base pairs as well as the clustering of polymorphisms in ITS1 in the *E. laidlawi* specimens indicates that this region is comparatively unstable. Slippage events might occur more often than in other regions and it is likely that the observed polymorphisms arose from two slippage events rather than one-by-one through point mutations. Thus, we propose that the 11 differences observed between

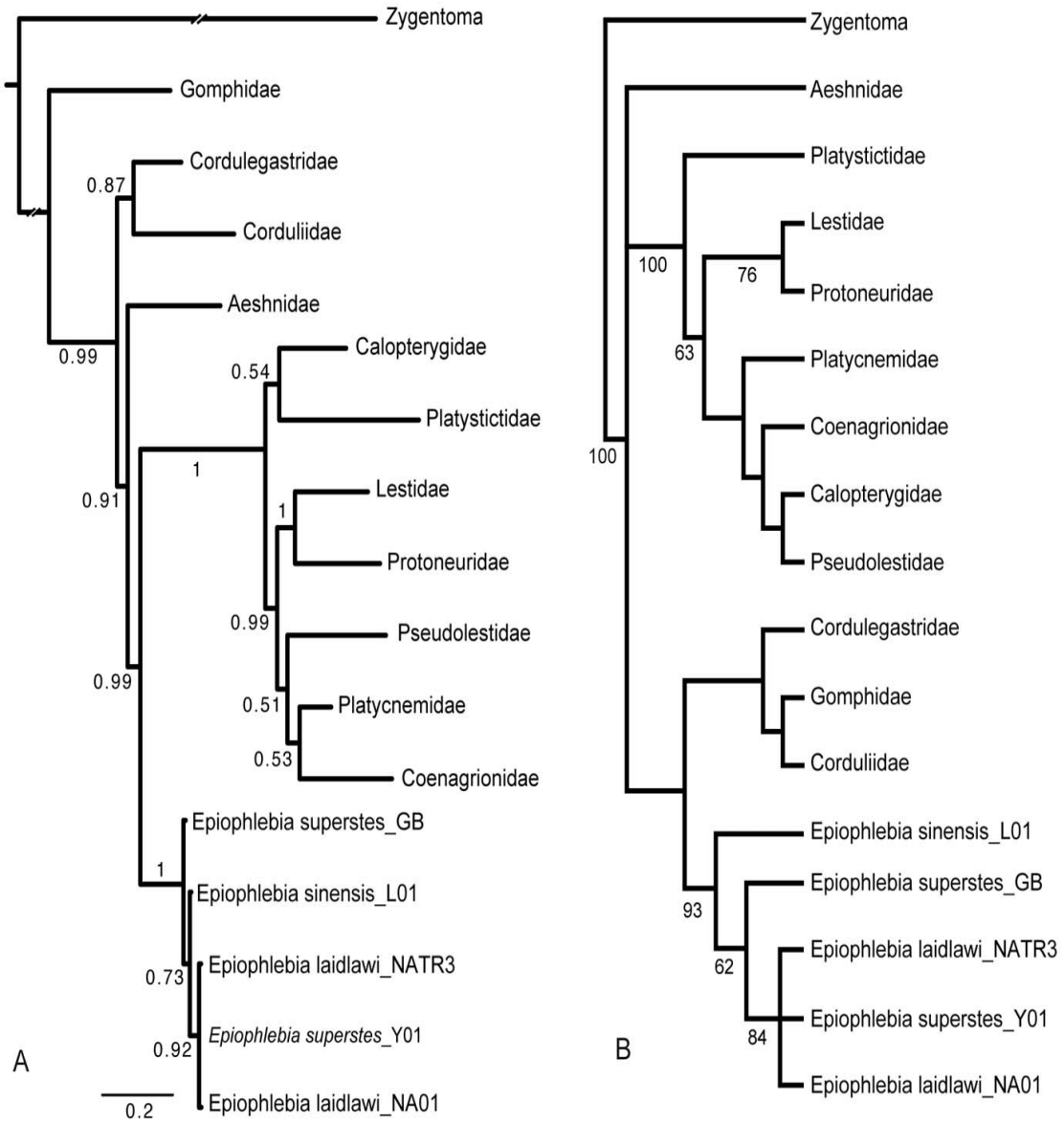

Figure 2. Results of phylogenetic analyses. (A) Phylogram of bayesian analysis of full dataset (see text). Numbers indicate posterior probability for respective branches. (B) Strict consensus of three trees from maximum likelihood analysis. Numbers indicate bootstrap values. Where these values are missing the respective node collapsed in the bootstrap analysis.
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E. laidlawi and *E. superstes* can be traced back to only four different events.

Even though there are so few variations between the specimens investigated we even observed a few different positions between the *E. superstes* sequences from GenBank that was used for comparison and the newly produced sequences. The specimens that were sequenced for the GenBank data are from a population in the vicinity of Tokyo, whereas our specimens are from Hokkaido in the far north of Japan. Therefore the observed differences might just reflect the sequences' origin form different populations and also indicate that there is not very much gene exchange between these populations.

The comparisons of sequences of different populations and of closely related species (Table 1) reveal that the interspecific variability in DNA sequences within different populations of Odonata can be much higher than between specimens of *Epiophlebia*. In total this suggests that gene transfer between its species and populations took place in the not so distant past.

Phylogenetic Analysis

Despite the differences in the general topology of trees generated with maximum parsimony, maximum likelihood and bayesian algorithms the specimens of *Epiophlebia* are always grouped in one well-supported clade but never appear in the arrangement of the currently recognized species. This arrangement, with specimens of *Epiophlebia* freely mixed on the tree, is independent of the composition of the dataset and of the algorithm applied. Even the fairly variable CO- and ITS-sequences produce this result when analysed separately. These results challenge the assumption of three separate extant species of *Epiophlebia*. Further investigations especially of the morphology should be done to clarify the variability of discriminating features and the taxonomic status of the three species.

Table 1. Inter- and intra-specific variation in different species of Odonata for the sequences investigated.

Sequence	Species/Populations	Accession No.	No. of mutations/length of sequence	Max. no. of mutations/length of sequence between *Epiophlebia* specimens
18S	*Enallagma parvum/E. nigridorsum*	AJ420939/AJ420938	3/1856	0/240
28S	*Orthetrum albistylum/O. triangulare*	AB127411/AB127410	6/603	0/1002
CO2	*Lestes sponsa/L. temporalis*	AB446428/AB446429	28/282	0–1/282
CO2	*Ischnura asiatica*	AB446399/AB446400/AB446401	4/283	
ITS2	*Calopteryx splendens/C. maculata*	AJ308363/AJ459198	25/253	4/265
ITS2	*Calopteryx haemorrhoidalis*: Italy/Morocco	AJ308348/AJ308347	3/213	
ITS1	*Anax panybeus/A. guttatus*	AB601902/AB601901	11/261	11/215*
ITS1	*Cordulia aenea*	AY274516/AY274535/AY274537/ AY274539	14/283	

*a total of 11 polymorphic nucleotide positions which probably arose from only two mutation events.
doi:10.1371/journal.pone.0038132.t001

Further results of the present phylogenetic analysis stating a non-monophyletic Anisoptera and a sistergroup relationship of *Epiophlebia* and Zygoptera are remarkable. Taxa for the analysis were originally selected to represent major clades from the phylogenetic system of Odonata as reconstructed in [8] and we expected to recover its general topology. However, our taxon sample is not as comprehensive as in any of the investigations that recently confirmed monophyletic Anisoptera with a sistergroup *Epiophlebia* [6,8,10,11]. Therefore, the differing topology found in the present analysis may well be attributed to effects of the composition of taxa in the dataset. Monophyletic Anisoptera as well as its sistergroup relationship with *Epiophlebia* are also supported by morphological characters found in extant as well as in fossil Odonata [3,29,30]. These relationships seem to be more probable than the topology found in our analyses, since our dataset was not compiled to be especially informative in respects of the relationships of high-level taxa of Odonata. However, since our results are reproducible with different algorithms and from different combinations of sequence data, they should be understood as a strong indication that higher level phylogenetic relationships within Odonata might not yet be finally resolved.

Biogeographic History of *Epiophlebia*

Firm biogeographic connections between the area of the present Himalayas, the Asian mainland and of Japan in former times are well documented by the Sino-Japanese floristic region [31]. However, since when an effective isolation of the Asian mainland and the Japanese populations is established, can only be estimated.

Presently Japan is separated from the mainland by sea-straits with depths of ca. 55 m north of Hokkaido and ca. 130 m between the southern island Kyushu and Korea [32]. The last substantial land bridge to Hokkaido was present during the Würm sea level lowering approximately 20,000 years BP [33]. According to some authors [32,34,35] a land bridge also existed between Kyushu and the Korean peninsula during this time (Figure 3). Assuming that during this period was the most recent possibility for genetic exchange between the Japanese and the mainland populations of *Epiophlebia*, it also follows that their distribution was significantly different from the present one.

Epiophlebia larvae apparently are very stenoecious and inhabit only cool headwaters of streams. During the last glacial maximum suitable environmental conditions probably were present throughout the lowlands south, southeast and east of the Himalayas. The area with adequate environmental conditions probably reached at least northwards to the connection between the southern Japanese island Kyushu and the mainland (Figure 3B). When the temperatures rose again at the end of the last ice age *Epiophlebia* retreated to the cooler higher areas. Consequently, the extant populations of *Epiophlebia* are small relicts of a formerly much larger and wider distributed population.

Previously the extant distribution of *Epiophlebia* was dated back to the Jurassic when Pangäa broke apart [18,21]. This might be true for the general Asiatic distribution of the ancestors of *Epiophlebia*. However, our data show that the extant species and most likely the genus are very much younger. Therefore, the interpretation that even the species go back to Jurassic time [21] can not be maintained.

Current climatic development threatens the existence of these populations [36]. With globally rising temperatures suitable habitats for species like *Epiophlebia* with such narrow tolerances for environmental parameters are in great danger of extinction.

Status of *Epiophlebia* Species

In the light of the extreme similarity of sequences in *Epiophlebia* specimens from Japan and Nepal, the observed morphological differences between their adults as well as their larvae tend to appear as minor local variations. In summary, this indicates that probably the Himalayan and the Japanese populations are in fact representatives of a single biological species. However, with the current climatic and topographic situation the chances of future genetic exchange between these populations are bleak. So, even if at present the different populations just represent one biological species, the probability that they are just on the verge of becoming "real" species is very high.

One might even assume that the populations in the different valleys of the Himalayas are separated from one another by the high mountain ranges as effectively as any of them is separated from the Japanese population by sheer distance [19]. So, perhaps even here there is a certain probability for the future formation of individual species.

For *E. sinensis* the situation is not very different. Only the amount of available data for this species is much worse than for the Japanese and the Himalayan populations. No larvae are known so far and sequence data are also sparse. Furthermore, the available information on the morphology is based on no more than two male specimens [18]. So, we know nothing about morphological

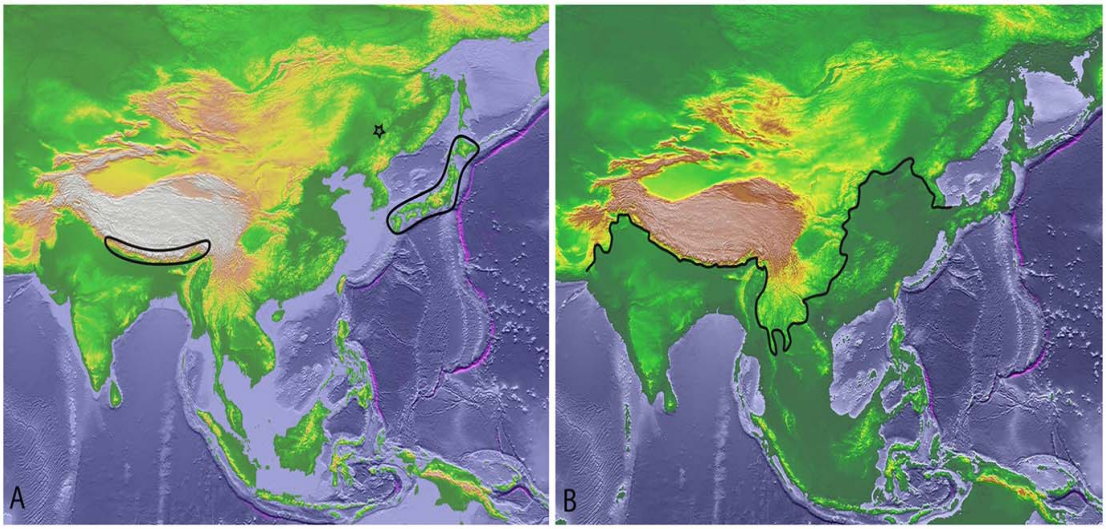

Figure 3. Distribution of *Epiophlebia*. (A) Known present distribution, the star marks the type locality of *E. sinensis* Li & Nel, 2011. (B) Approximate coastline during the Würm glacial period with land bridges between Japan and the mainland. The black line marks a possible northern boundary of the range of *Epiophlebia* during this time. (Modified from [47]).
doi:10.1371/journal.pone.0038132.g003

variability. The available sequence data are, however, as similar to those of *E. superstes* as those from *E. laidlawi* are. If our biogeographic interpretations are correct, than it even seems to be probable that genetic exchange between *E. superstes* and *E. sinensis* was possible until a more recent time than between *E. superstes* and *E. laidlawi*. Thus, *E. sinensis* might also be just another population of this single species of *Epiophlebia*.

Mutation Rates of Different DNA-sequences of *Epiophlebia*

The analyses of the different sequences indicate a low variability even in quickly evolving parts of the genome as in the ITS genes. This implies that the range expansion of *Epiophlebia* during the last glacial maximum was very quick, because these circumstances enhance low genetic diversity in a population [37].

On the other hand, this might present an opportunity to estimate mutation rates backwards, at least for the highly variable sections of the DNA. Assuming that the genome was very homogeneous after range expansion during the last glacial maximum about 20,000 years ago, the substitution rate can be inferred from the number of differences in the sequences that we were able to acquire for *Epiophlebia*:

For ITS1 a rate of 4.65×10^{-7} to 2.56×10^{-6} substitutions per site per year can be deduced, for ITS2 there are 5.19×10^{-7}, and for CO2 1.89×10^{-7} substitutions per site per year. Compared to such rates in other organisms [38,39] the rates for *Epiophlebia* are clearly among the higher values. Nevertheless, these figures do not contradict the assumption of a comparatively recent separation of the *Epiophlebia* populations.

Materials and Methods

Eleven larvae of *Epiophlebia superstes* (Sélys, 1889) of different instars were collected in 2010 in Hokkaido, Japan, fixed and stored in 80% ethanol.

Twelve larvae of different instars of *E. laidlawi* Tillyard, 1921 were collected in 2008 and 2009 in Nepal, fixed in 4% formaldehyde and stored in 70% ethanol. Specimens are stored in the collection of the Hindu Kush Himalayan Benthological Society, Nepal.

Two adults of *E. sinensis* Li and Nel, 2011 were collected in 2011 in Heilongjiang province, China as described in [18]. Two femora of these specimens were available for sequencing.

Larvae of *Coenagrion* spec. were collected in the botanical garden of the Georg-August-University in Göttingen, Germany; fixed in FAE and stored in 70% ethanol (to simulate the conditions of preservation of the other specimens).

For detailed morphological investigation tomography data of four larvae of *E. laidlawi* and three specimens of *E. superstes* were acquired at the Swiss Light Source synchrotron (SLS, Viligen, Switzerland, proposal no. 20100088 by TH) and with a v|tome|x s X-ray scanner (GE Sensing & Inspection Technologies GmbH phoenixjx-ray) at the Palaeontological Institut at University Bonn (Germany).

Contamination Prevention

The DNA analysis was carried out under strict safety conditions [24], such as separation of pre- and post-PCR laboratories and the use of disposable protective clothing, glassware, and disposable gloves. All experiments took place with disposable laboratory ware, such as pipette tips and cups, while workbenches and other laboratory equipment were cleaned with detergents (Alconox^{TM} Detergent, Aldrich, Germany), bi-distilled water, and ethanol before use for each sample to avoid cross-contamination. In accordance with the recommendations of [40], all disposable ware and solutions, buffers, and $MgCl_2$ were irradiated with ultraviolet light at a short distance employing aluminum foil coating. Negative PCR and extraction controls were employed.

DNA Extraction

Before DNA extraction the guts of the specimens were removed to avoid possible contamination with foreign DNA. Only thorax and leg muscles were used for the analyses.

For cell lysis, 200 µl ATL buffer (Qiagen, Germany) was added to 10–20 mg of tissue. The mixture was homogenized in a TissueLyser (Qiagen) at 30 Hz for 60 s using a 5 mm steel ball. After removal of the ball, 30 µl of Proteinase K (20 mg/ml) was added to the solution and incubated at 56°C for 18 hours under constant agitating. 200 µl of the supernatant were used for

automated DNA extraction with the Biorobot® EZ1 (Qiagen, Germany) following the protocol of the QIAamp DNA FFPE Tissue procedure. The elution volume was 50 µl; the DNA extract was stored at −20°C. We carried out at least two independent DNA extractions and sequencings for each individual to permit authentication of the analysis results by means of comparison. Heterogeneity was only detected in ITS1 of *E. laidlawi*.

Primer Design

Due to storage conditions and influences of the preservatives, DNA of the *E. laidlawi* specimens was degraded. Therefore, primers were designed matching the profile of ancient DNA characteristics [24] and amplifying fragments between 200 and 300 bp each (Table S2). Because of this limitation, we amplified only polymorphic sites instead of the whole genes in the cases of the mainly conserved 18 S and 28 S rRNA genes [8]. To gain information on the polymorphic sites, alignments of sequences from GenBank of numerous different species were carried out using MegAlign (Lasergene, www.DNASTAR.com) and the Clustal V algorithm. The primers were designed to discriminate against human DNA and to amplify as many taxa of Odonata as possible.

PCR Parameters and Sequencing

The reaction volume in each setting was 25 µl, containing 12.5 µl 2x master mix (AmpliTaq® Gold 360, ABI), 0.4 µM of each primer, 5–7.5 µl of DNA extract and filled up with RNAse free water (Qiagen). PCR was carried out under the following conditions: initialization 95°C for 5 min; 40 – 45 cycles at 95°C for 1 min, *annealing temperature* (Table S2) for 1 min, 72°C for 2 min; final elongation at 72°C for 7 min; and soak at 10°C for 10 min. The PCR success and product quantity were checked by agarose gel electrophoresis. Further purification and sequencing were carried out with commercial kits (MiniElute® PCR Purification Kit, Qiagen, ABI Prism BigDye V 3.1 Terminator Cycle Sequencing Kit and NucleoSeq Kit, Macherey-Nagel) as specified by the manufacturers.

Both the forward and reverse primers used for amplification were also used for the sequencing reaction. The sequencing conditions were: initial at 96°C for 10 min; 25 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. For sequencing an ABI 310 genetic analyser with POP6 polymer was used. Sequence reads were checked for quality and assembled using SEQMAN (Lasergene; www.DNASTAR.com).

Phylogenetic Analysis

Sequences were compiled and aligned using *MEGA* version 5 [41]. Ribosomal DNA sequences were aligned automatically with the clustalW algorithm and standard parameters. Protein coding mitochondrial sequences were aligned manually via the corresponding amino acid sequences. For alignment of ITS sequences the online version of MAFFT [27,42] was used.

The most appropriate models of DNA substitution for bayesian and maximum likelihood tree searches were selected with MrModeltest 2.3 [43] and PAUP*4.0 b10 [44] separately for each sequence alignment and for a combined dataset containing all sequences except CO1, which was not used in the analysis since it was not possible to obtain sequences of this gene from *E. laidlawi* or *E. sinensis* specimens. The complete dataset contained 17 taxa, including a representative of Zygentoma as outgroup, and 4799 positions. Taxa from Odonata were chosen to represent all major clades as present in the phylogeny of [8]. A Nexus file of the alignments are provided as Dataset S1.

To find the most probable phylogenetic relationships of the *Epiophlebia* species the aligned sequences were combined into a mixed dataset for analysis with MrBayes 3.1.2 [45,46]. MrBayes offers the unique possibility to reconstruct a phylogeny based on information form several different genes in a single analysis, while applying the most appropriate model to each gene sequence. Additional tree searches were done for the combined dataset with global parameters with the maximum likelihood (ML) algorithm in PAUP* and for each gene individually with MrBayes and with ML in PAUP*. To check node stability for ML a bootstrap analysis with 1000 replicates was done with the same parameters as for the original analysis.

Supporting Information

Figure S1 Alignment of CO2 sequences from different specimens of *Epiophlebia* species. *E. superstes*_GB = reference sequence from GenBank.
(TIF)

Figure S2 Alignment of ITS2 sequences from different specimens of *Epiophlebia* species. *E. superstes*_GB = reference sequence from GenBank.
(TIF)

Table S1 GenBank accession numbers for sequences used in phylogenetic analysis. * = this paper.
(DOC)

Table S2 Primers. # this paper; * [48]; *primers in italics* are specific only for *Epiophlebia*.
(DOC)

Dataset S1 Data matrix in NEXUS format including parameters for bayesian and maximum likelihood analysis.
(NEX)

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Author Contributions

Conceived and designed the experiments: SB PvG SH DNS RDTS KY TH. Performed the experiments: SB PvG TH. Analyzed the data: SB PvG TH. Contributed reagents/materials/analysis tools: PvG DNS RDTS JL XZ KY. Wrote the paper: SB PvG TH SW.

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Figure S1 Alignment of CO2 sequences from different specimens of Epiophlebia species. E. superstes_GB= reference sequence from GenBank

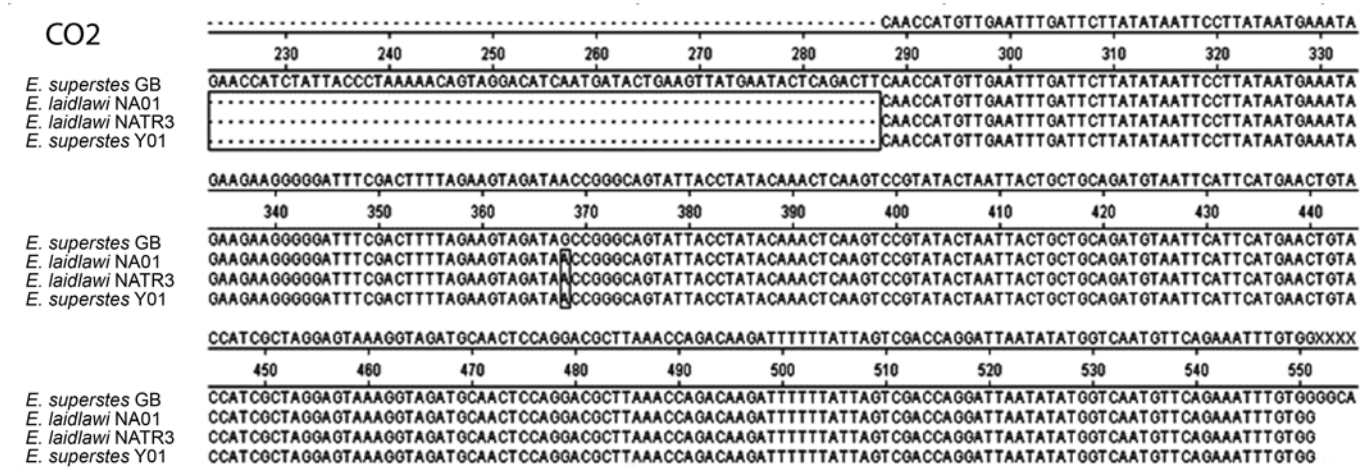

Figure S2 Alignment of ITS2 sequences from different specimens of Epiophlebia species. E. superstes_GB= reference sequence from GenBank.

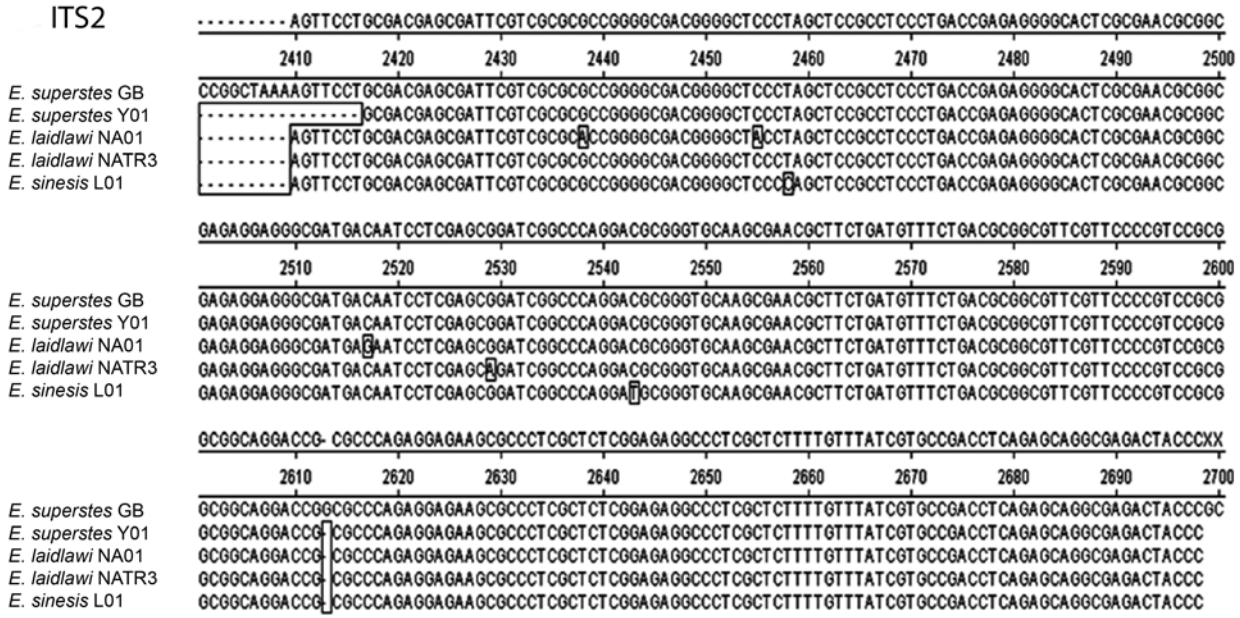

Table S1. GenBank accession numbers for sequences used in phylogenetic analysis. * = this paper.

		18s rDNA	28s rDNA	ITS1	ITS2	CO2
Odonata: Zygoptera: Hemiphlebiidae	*Hemiphlebia mirabilis*	FN356183				
Zygentoma	*Tricholepidion gertschi*	AF370789				NC_005437
Zygentoma	*Tricholepidion* sp.		AY338685			
Zygentoma	*Ctenolepisma longicaudata*			AY210810	AY210810	
Odonata: Zygoptera: Calopterygidae	*Calopteryx aequabilis*					EU055325
Odonata: Zygoptera: Calopterygidae	*Calopteryx splendens*	DQ008208				
Odonata: Zygoptera: Calopterygidae	*Calopteryx amata*		FJ009949	AJ308361	AJ308361	
Odonata: Zygoptera: Platycnemidae	*Platycnemis pennipes*	DQ008207	FJ009928	AJ459230	AJ459230	EU055397
Odonata: Zygoptera: Lestidae	*Lestes* sp.					EU055330
Odonata: Zygoptera: Lestidae	*Chalcolestes viridis*	AJ421949	EU424331	AJ421949	AJ421949	
Odonata: Zygoptera: Protoneuridae	*Protoneura capillaris*	EU055184	EU055282			EU055381
Odonata: Zygoptera: Platystictidae	*Palaemnema melanostigma*	FN356142	EU055317	FN356142	FN356142	EU055415
Odonata: Anisoptera: Gomphidae	*Gomphus* sp.					EU055338
Odonata: Anisoptera: Gomphidae	*Gomphus vulgatissimus*	FN356091	FJ712320	FN356091	FN356091	
Odonata: Anisoptera: Cordulegastridae	*Cordulegaster dorsalis*					EU055376
Odonata: Anisoptera: Cordulegastridae	*Cordulegaster boltonii*	FN356072	FJ596634	FN356072	FN356072	
Odonata: Anisoptera: Aeshnidae	*Anax parthenope*					DQ166787
Odonata: Anisoptera: Aeshnidae	*Anax imperator*	FN356035	FJ596632	FN356035	FN356035	
Odonata: Zygoptera: Pseudolestidae	*Pseudolestes mirabilis*	EU055220	EU055318			FJ606784
Odonata: Anisoptera: Corduliidae	*Hesperocordulia berthoudi*					EU055357
Odonata: Anisoptera: Corduliidae	*Idionyx imbricata*	EU055189	EU055287			
Odonata: Anisoptera: Corduliidae	*Idionyx optata*			FN356096	FN356096	
Odonata: Zygoptera: Coenagrionidae	*Ischnura elegans*			FN356103	FN356103	HQ834805
Odonata: Zygoptera: Coenagrionidae	*Ischnura barberi*	EU055136	EU055231			
Odonata: Zygoptera: Heliocharitidae	*Heliocharis amazona*	AJ746326		AJ746326	AJ746326	
Odonata: "Anisozygoptera"	*Epiophlebia superstes*	FN356086	EU424328	FN356086	FN356086	EU055421
Odonata: "Anisozygoptera"	*Epiophlebia superstes**	JQ943599	JQ943588 (S1), JQ943589 (S2), JQ943590 (S3), JQ943591 (S4	JQ943606	JQ943609	JQ943603
Odonata: "Anisozygoptera"	*Epiophlebia laidlawi** NATR3	JQ943597			JQ943611	JQ943605
Odonata: "Anisozygoptera"	*Epiophlebia laidlawi** NA01	JQ943598	JQ943592 (S1), JQ943593 (S2), JQ943594 (S3), JQ943595 (S4)	JQ943607	JQ943610	JQ943604
Odonata: "Anisozygoptera"	*Epiophlebia sinensis**			JQ943608		

Table S2. Primers.

System	Primer	Sequence (5`-3´)	Length of resulting sequence(bp)	Annealing temperature
18S rDNA	18S_up#	GGTTCCTTGGATCTTACCCACACT	240	59°C
	18S_low#	GCAGAACCTACCATCGAAAGTTGAT		
28S rDNA-1	28S_up#	TCGGACACGCTCCGCTAAAC	191	64°C
	28S_low#	GCCAGGCATAGTTCACCATCTTTC		
28S rDNA-2	28S2_up#	CCGGTAAAGCGAATGATTAGAG	267	60°C
	28S2_low#	CCACCGTCCTGCTGTCTTAA		
28S rDNA-3	28S3_up#	GGAATCCGCTAAGGAGTGTGTAA	251	58°C
	28S3_low#	AGGGCCTCGCTGGAGTATTT		
28S rDNA-4	28S4_up#	CCGTTGCACACGAGTCAGTC	293	58°C
	28S4_low#	TCGCGTTCCAAACCCTATCT		
ITS1	*ITS1_up*	*CCTGCGGAAGGATCATTATTGT*	*215*	*56°C*
	ITS1_low	*GGTGGGTTCAGGGGAGAGAC*		
ITS2	*ITS2_up*	*AGTTCCTGCGACGAGCGATT*	*289*	*62°C*
	ITS2_low	*GGGTAGTCTCGCCTGCTCTGA*		
CO2	CO2_up	TCAACCATGTTGAATTTGATTCTTAT	265	55°C
	CO2_low	CCACAAATTTCTGAACATTGACC		
CO1	CO1_up*	GGATCACCTGATATAGCATTCCC	500	50°C
	CO1_low*	CCCGGTAAAATTAAAATATAAACTTC		

this paper; *[40]; *primers in italics* are specific only for *Epiophlebia*.

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#NEXUS

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        FORMAT DATATYPE = DNA GAP = - MISSING = ?;
        MATRIX
        Zygentoma
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C---GT-
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TCTGGG-GGCCGAGGGAGGACGGAGCGG----------GTCCCCTCGCGGGGACGGC-TCC-CGCACTCCCGGGGCGTC---TGATTCCTC--ATCGCGAGGGAAGGCGC--
ACCCAGAGCGCACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGC???????????????????????????????????????????????????????????????????????????????????????????????????
???????????????????????????????????????????????????????????????????????????????????CCGGTAAAGCGAATGATTAGAGGCCTTGGGGCCGAAACGACCTCAACCTATTCTCAAACTTTAAA
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AGGACGGTGGGGAATCCGCT-----------AAGGAGTGTGTAAC--AACTCACCTGCCGAAGCAACTAGCCCTGAAAATGGATGGCGCTGAAGCGTCGTGCCTATACCCGGCCGTCGCGGCAGGGCGGACCCCCCTC----------
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------------------------CCGTTGCACACGAGTCAGTCGATCCTAAGCCCTAGGAGAAATCCGATACTAACGGCGATGGGAAAT-----ACTTTTTTCTGAGACGGA-----------------
AGCCCATTGATCGGGCGAAAGGGAATCCGGTTC-CTATTCCGGAACCCGGCAGCGGAACCGT----------------TGTTTGTCGGG-
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---------------------------------------GCGACGAGCGATTCGTCGCGCGCCGGGGCGACGG---------------------GGCTCCCTAGCTCCGCCTCCCTGACCGAGAGGGGCA--------------
CTCGCGAACGCGGCGAGAGGAGG-------------------------
GCGATGACAATCCTCGAGCGGATCGGCCCAGGACGCGGGTGCAAGCGAACGCTTCTGATGTTTCTGACGCGGCGTTAAGGAGGCAACGCGGAGGGATTTTTCGG-ACGGCGGGTACCTGGCGACCT---------------------
---CGGGTTTTCAAAGACGTTCGCCCGCC---------GGAAGGAACGCCCGCGTCCGCGCCCTCTCTCTTT-TTTCTTCCCGTCTCTCCCCTGAACCCACC
       Epiophlebia_superstes_gb
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GAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATACGGGACTCATCCGAGGCCCCGTAATCGGAATGAGAACACTTTAAATCC
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CATTTGTTGGTTTTCA-
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GCTTCAGCTTGGATGGCTACCCGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATGCGCGCGAGTCGTTGGGTCGGACTCGCTCCGCTCAAACCCATAGGCGCAATGAAAGTGAAGGCCACCT----TCTGGG-
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ACCCAGAGCGCACACGTTGGACCCGAAAGATGGTGAACTATGCCTGGCCAGGACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAGCTGGTATAGGGGCGAAAGACTAATCGAAC
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AGACGCCGTCGGGAGCCCCGAAAGAGTTTTCTTTCTGCATGAGCGTTCGAGTTCCCTGGAATCCCTCTCGCAGGGAGATAGGGTT-
TGGAACGCGAAGAGCACCGCAGTTGCGGCGGTGCCCGGGAATTCCCGACGGACGAGCGATTCGTCGCGCGCCGGGGCGACGG----------
GGCTCCCCTAGCTCCGCCTCCCTGACCGAGAGGGGCA----------CTCGCGAACGCGGCGAGAGGAGG----------
GCGATGACAATCCTCGAGCGGATCGGCCCAGGACGCGGGTGCAAGCGAACGCTTCTGATGTTTCTGACGCGGCGAGGAGGATTTTTCGG-ACGGCGGGTACCTGGCGACCT----------
---CGGGTTTCAAAGAGTTCGCCCGCC----------GGAAGGAACGCCCGCGTCCGCGCCCTCTCTCTTT-TTTCTTCCCGTCTCTCCCCTGAACCCACC
      Epio_sinensis
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---------GGCTcCCCAGCTCCGCCTCCCTGACCGAgAgGGGCA--------------CTCGCGAACGCGGCGAGAGGAGG-------------------------
GCGATGACAATCCTCGAGCGGATCGGCCCAGGATGCGGGTGCAAGCGAACGCTTCTGATGTTTCTGACGCGGCGTTAAGGAGGCAACGAGGAGGGATTTTTCGG-ACGGCGGGTACCTGGCGACCT---------------------
---CGGGTTTTCAAAGACGTTCGCCCGCC---------GGAAGGAACGCCCGCGTCCGCGCCCTCTCTCTTTCTTTCTTCCCGTCTCTCCCCTGAACCCACC
       Calopterygidae
CAAACAATCGAAACCTACTTGACGGACAAAAAATTGAAACAGCCTGAACCATTCTACCAGTATTTGTATTAATCATAATTGCCATACCATCTCTTCGTCTACTATACTTAATAGATGAAATCAGAGAACCATCAATTACTTTAAAAA
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CCTTGGTGACTCTGAATAACTTTTGGCTGATCGCACGGTCGTTGCACCGGCGACGCATCTTTCAAATGTCTGCCTTATCAACTTTCGATGGTAGGTTCTGCGCCTACCATGGTTGTAACGGGTGACGGGGAATCAGGGTTCGATCCCG
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G?CGGAGGTGTGTCTCGGACGGTCGGTTCGCCGCTTGTCGGTGCTCAACTGGCCGGT-CCGGACGTCCTGCCGGTGGGGCCGC-AA--
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CATTTTGTTGGTTTTCA-
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AAGCTCTTGGGTTCCGGGGGAAGTATGGTTGCAAAGCTCAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACAC?????????????????????????????????????????????
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GCTACGGCTTGGATGGTCCACCCGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATGCGCGCGAGTCGTTGGGTCGGACACGCTCCGCT-AAACCCATAGGCGCAATGAAAGTGAAGGCCGCCT----TCTGG-
TGGCCGAGGGAGGACGGGGTCGTTC----GCGCGCTCCGGCGCGCGTCGG--TCCCCGCACCCCCGGGGCGTC---TGATCCCTC--ATCGCGAGGGGGAGGCGC--
ACCCAGAGCGCACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGCCAGGACGAAGTCAGGGGAAACCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAGCTGGGTATAGGGGCGAAAGACTAATCGAAC
CATCTAGTAGCTGGTTCCCTCCGA--AGTTTCCCTCAGGATAGCTTGCGCTCGGAGGA----------------
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       Platycnemidae
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GTCGGAGGTGTGTCTCGGACGGTCGGTTCGCCGCTTGTCGGTGCTCAACTGGCCGGT-CCGGACGTCCTGCCGGTGGGGCCGC-GA--
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GCTAC?????GGATGGTCCACCCGACCGTCTTGAAACACGGACCAAGGAGTCTAACATGCGCGAGTCGTTGGGTCGGACACGTCCGCT-AAACCCATAGGCGCAATGAAAGTGAAGGCTGCCG----TCCGG-
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GGAGACGAGGGGAAGAAATTCCGGTCGGTTC----------TCCTGGGAAAAGATT
Lestidae
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      Cordulegasteridae
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Corduliidae
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ACGAGAGAGGGAAAGATTTTTCCCGTCGGTTC---------------ATTCCGAAGAAAAGACT
;
END;

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END;

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       CODESET * UNTITLED   =  universal:  538 -  4799, mtdna.dros:  1 -  537;
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       charset COII_1st = 1 - 537 \ 3;
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       charset COII_3st = 3 - 537 \ 3;
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       charset 28s = 2238 - 4394;
       charset ITS2 = 4395 - 4625;
       charset ITS1 = 4626 - 4799;
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       prset applyto = ( all ) ratepr = variable;
END;
```

3. Homologization of the Flight Musculature of Zygoptera (Insecta: Odonata) and Neoptera (Insecta)

3.1. Contribution to this Publication

Conceived and designed the experiments: SB CG TH.
Performed the experiments: SB CG.
Analyzed the data: SB CG TH.
Contributed reagents/materials/analysis tools: SB CG TH.
Wrote the paper: SB TH.

3.2. Publication

Büsse S, Genet C & Hörnschemeyer T (2013) Homologization of the Flight Musculature of Zygoptera (Insecta: Odonata) and Neoptera (Insecta). PLoS ONE 8(2): e55787. doi:10.1371/journal.pone.0055787

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Homologization of the Flight Musculature of Zygoptera (Insecta: Odonata) and Neoptera (Insecta)

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Abstract

Among the winged insects (Pterygota) the Dragonflies and Damselflies (Odonata) are unique for several reasons. Behaviourally they are aerial predators that hunt and catch their prey in flight, only. Morphologically the flight apparatus of Odonata is significantly different from what is found in the remaining Pterygota. However, to understand the phylogenetic relationships of winged insects and the origin and evolution of insect flight in general, it is essential to know how the elements of the odonatan flight apparatus relate to those of the other Pterygota. Here we present a comprehensive, comparative morphological investigation of the thoracic flight musculature of damselflies (Zygoptera). Based on our new data we propose a homologization scheme for the thoracic musculature throughout Pterygota. The new homology hypotheses will allow for future comparative work and especially for phylogenetic analyses using characters of the thoracic musculature throughout all winged insects. This will contribute to understand the early evolution of pterygote insects and their basal phylogenetic relationship.

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Within the insects the Odonata arguably are the group with the most impressive flight skills (e.g. [1]). Each wing pair can be controlled independently and some species are even able to fly backwards [2]. Through these flight skills Odonata are the avian key predators among insects [1].

The unique flight abilities are also reflected in a unique morphology. The meso- and metathorax forms a functional unit, the ptero- or synthorax, which is tilted caudally by 45°. The pleurites are strongly enlarged in dorso-ventral direction, whereas, the tergites and sternites are unusually small if compared to other pterygotes [2–4].

The muscles responsible for the wing movement are connected via cap tendons and sclerites directly to the wings [5]. This exclusively direct mechanism of wing movement distinctly sets Odonata apart from all other winged insects; where the wing beat is done mainly through a system of indirect muscles, many of which are highly reduced or missing in the Odonata (e.g. [6]).

Several publications address the structures of the flight apparatus of Odonata [5,7–10], the aerodynamics of odonatan flight [10–12], the mechanics [2] and function of the flight musculature and the mechanoreceptors of the wing [10] as well as the complexity of the wing venation [13]. All these publications deal mainly with representatives of Anisoptera. In total, the knowledge about the odonatan thorax morphology shows a distinct deficit for the Zygoptera, which we, therefore, focused our comparative investigation on.

Major research has been carried out by Asahina [7], who studied *Mnais strigata* Hagen, 1853 (Zygoptera), *Davidius nanus* (Sélys, 1869) (Anisoptera) and *Epiophlebia superstes* Sélys, 1889 (*Epiophlebia*). Ninomiya and Yoshizawa [14], investigated the skeletal morphology of *Coeliccia ryukyuensis ryukyuensis* Asahina, 1951 (Zygoptera), *Tanypteryx pryeri* (Sélys, 1889) (Anisoptera) and *Epiophlebia superstes*.

Presently there seems to be widespread agreement on ground pattern hypotheses for the wing base sclerites and for the flight musculature in Neoptera [15–18]. Even homologies between Ephemeroptera and Neoptera are mainly resolved [17,19], while hypotheses on the homologies between Odonata and the remaining Pterygota are still under discussion [17,19,14,10].

The aim of our comprehensive comparative investigation of the flight musculature of the Zygoptera is to identify variabilities among the Zygoptera and to establish homology hypotheses for the thoracic musculature of Odonata and Neoptera.

Results

In the following descriptions of the musculature the condition in *Phyrrhosoma nymphula* (Fig. 1, 2, 3, 4, 5, 6, 7, 8) is used as a point of reference. This infomaration is supplemented with and compared to data from *Coenagrion puella*, *Enallagma cyathigerum*, *Ischnura elegans Calopteryx splendens* (Fig. 9, 10, 11, 12), *Platycnemis latipes*, *Platycnemis pennipes* and *Lestes viridis*.

Together with the description of the muscles found, we already present our homology hypothesis by using the muscle names as proposed for Neoptera by Friedrich & Beutel [18]. We are aware that this presents a mixture of description and interpretation. However, stricter separation of these aspects would not support a clear and easily understandable presentation of the results.

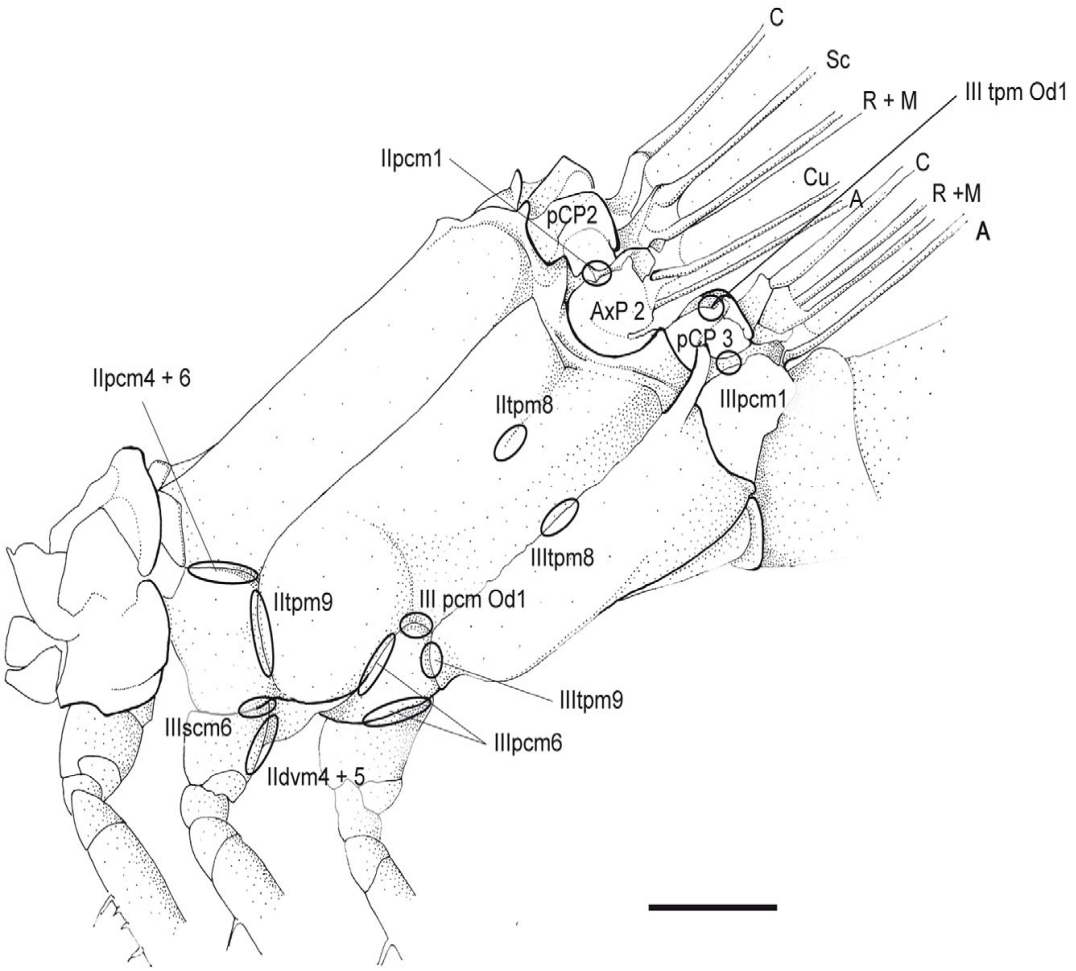

Figure 1. Thorax of *P. nymphula*, muscle attachment points as seen from outside, left, lateral view. Abbreviations: A – anal vein, AxP – axillary plate, C – costa, Cu – cubitus, M – media, pCP – proximale costal plate, R – radius, Sc – subcosta.
doi:10.1371/journal.pone.0055787.g001

For establishing our homology hypotheses we supplemented our data with information from the literature [7,10,14,17], focusing on Asahina's comprehencive study of *Epiophlebia superstes* [7], which represents a conspicuous mixture of anisopteran and zygopteran characters [4,7,20,21]. Furthermore, in many aspects *Epiophlebia* seems to have the most ancestral character distribution within the Odonata (e.g. [22]).

For the skeletal elements of the thorax the nomenclature by Asahina [7] is used. Where necessary, this is supplemented by Snodgrass [6] and Ninomiya and Yoshizawa [14].

The homologies as well as the presence or absence of each muscle are listed in Table 1. In the muscle descriptions Asahina's muscle numbers are given in square brackets after the name of each muscle. For mesothoracic muscles Asahina's numbers for the corresponding metathoracic muscles are added in parentheses. The muscles are listed due to their occurrence in the pterothorax, from anterior to posterior. An additional table comparing our results with data from several other publications is available as supporting information (Table S1).

Since the prothorax has no active role in flight, it is omitted in this study.

Musculature of the Pterothorax

In the following we describe 44 muscles, 19 muscles of the mesothorax and 23 muscles of the metathorax. Two previously undescribed muscles, M. mesopleura-scutalis proximalis (**IIItpm2**) and M. metapleura-scutalis proximalis (**IIItpm2**), are described for *P. nymphula*, *C. puella*, *I. elegans*, *E. cyathigerum* and *P. latipes*. The presence of these two muscles in *P. pennipes* could not be confirmed.

Musculature of the Mesothorax

IIpcm1 - M. mesanepisterno-trochantinalis [= muscle no. 21 in Asahina's nomenclature [7] (43 = corresponding muscle in metathorax)].

Origin: Preepisternum 2.

P. nymphula (Fig. 4), *C. splendens* (Fig. 10).

Insertion: Inserted with a long tendon at the anterior edge of proximale costal plate two (pCP2). The point of insertion is not exactly the edge but rather the membrane, which is connected with pCP2.

P. nymphula (Fig. 3, 4),

Characteristics: The muscle is short and thin and has a dorsal cap tendon. It is a direct tonic depressor muscle [10].

IIpcm2 - M. mesobasalare-trochantinalis [22 (44)].

Origin: Preepisternal apodem [7].

P. nymphula (Fig. 2), *C. splendens* (Fig. 9).

Insertion: Lateral to muscle IIpcm1 at the cranial edge of pCP2.

P. nymphula (Fig. 7E, 8B), *C. splendens* (Fig. 9).

Characteristics: It is a strong muscle with a dorsal cap tendon. In *Epiophlebia* [7] and in Anisoptera [17] this muscle was described

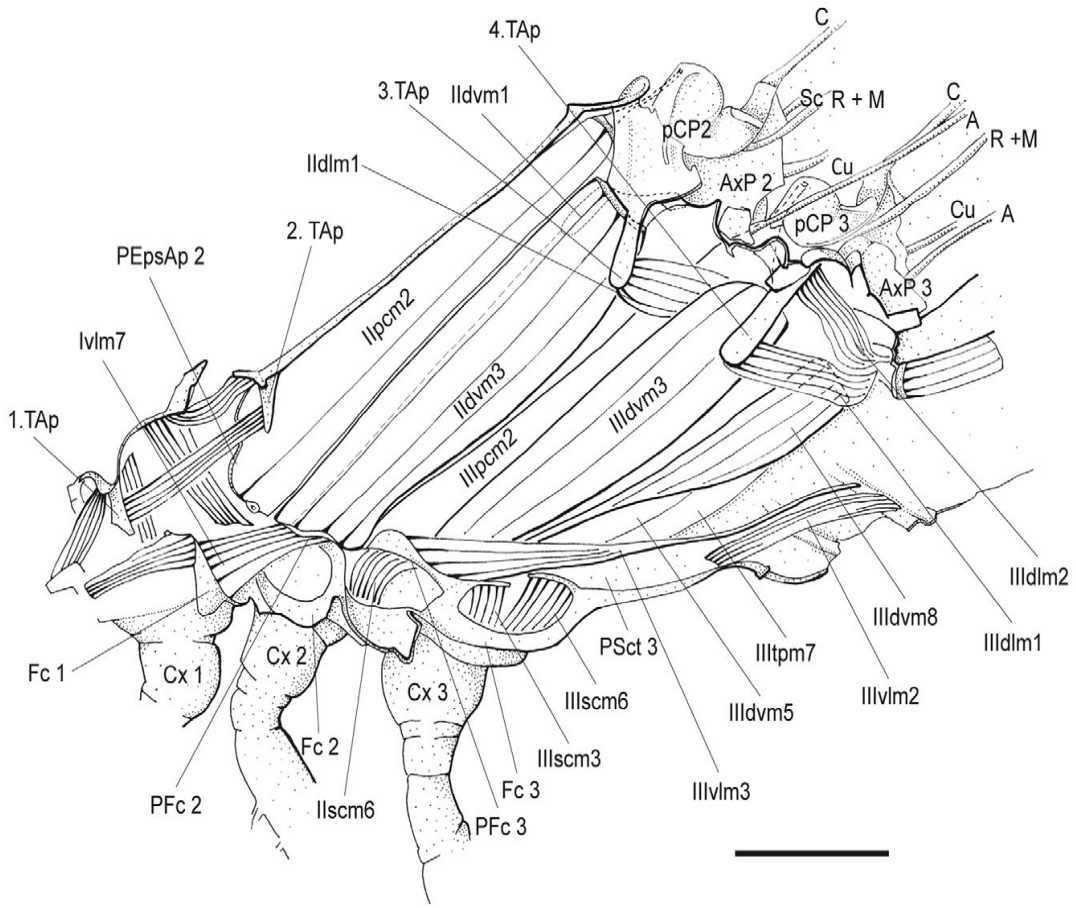

Figure 2. ***P. nymphula*****, innermost layer of thorax musculature, longitudinal cut, right.** Abbreviations: A – anal vein, AxP – axillary plate, C – costa, Cu – cubitus, Cx – coxa, Fc – Furca, M – media, pCP – proximale costal plate, PEpsAp – preepisternal apodem, PFc – prefurca, PSct – prescutum, R – radius, Sc – subcosta, TAp – tergal aphophyse.
doi:10.1371/journal.pone.0055787.g002

as dichotomous, which is not the case in the species investigated herein. It is a direct flightmuscle [10].

IIdvm1 - M. mesonoto-sternalis [23' (46')].

Origin: Distal, via a long tendon, at the preepisternal apodem to which IIdvm3 is attached.

P. nymphula (Fig. 2).

Insertion: At the tergum close to the tergal bridge, lateral of muscle IIdvm3.

P. nymphula (Fig. 2).

Characteristics: It is a short muscle distal from muscle IIdvm3. Its presence in *P. pennipes* could not be confirmed. The muscle is elongate in *P. latipes* compared to *P. nymphula*. In *C. splendens* it shows its maximal length.

IIdvm3 - M. mesonoto-trochantinalis posterior [23 (46)].

Origin: Ventral of the apodem that originates at the inner wall of prefurca 2.

P. nymphula (Fig. 2), *C. splendens* (Fig. 9).

Insertion: Via a tendon, lateral at the mesoscutum between the tergal cone and tergal bridge [10] or at the inner bar of the tergal articulation [5].

P. nymphula (Fig. 2), *C. splendens* (Fig. 9).

Characteristics: The muscle has a dorsal cap tendon. It is an indirect tonic depressor of the wing [10].

IIdvm4 - M. mesonoto-coxalis anterior [26 (48)].

Origin: Basal at the anterior part of the mesocoxa. In *C. splendens* more postero-lateral at the base of the mesocoxa.

P. nymphula (Fig. 1).

Insertion: Inserted with a tendon at the lateral part of the semi-detached scutal plate. In *C. splendens* at the upper edge of pCP2.

P. nymphula (Fig. 3), *C. splendens* (Fig. 10).

Characteristics: See muscle IIdvm5.

IIdvm5 - M. mesonoto-coxalis posterior [27 (49)].

Origin: Basal at the anterior part of the mesocoxa, postero-median of muscle IIdvm4. In *C. splendens* caudal of muscle IIdvm4.

P. nymphula (Fig. 1).

Insertion: With a tendon at the proximal edge of axillary plate two (AxP2), or at the membrane between the mesoscutum and AxP2.

P.nymphula (Fig. 3), *C. splendens* (Fig. 10).

Characteristics: The muscles IIdvm4, IIdvm5 and IItpm9 are direct flight muscles. IIdvm4 and IIdvm5 are tonic lifters and IItpm9 is a phasic depressor [10]. IIdvm4 and IIdvm5 have cap tendons and are distinctly smaller than IItpm9. The insertion in *C. splendens* is located laterally and more ventral than in *P. latipes*. In all species studied the origin of muscles IIdvm4 and IIdvm5 is also the attachment point of the coxal musculature.

IIdlm1 - M. prophragma-mesophragmalis [25 (45)].

Origin: Proximal end of the 3. tergal apophyse.

P. nymphula (Fig. 2), *C. splendens* (Fig. 10).

Insertion: Lateral at the posterior edge of the postnotum 2.

P. nymphula (Fig. 6A).

Characteristics: Broader than in *Epiophlebia* and in Anisoptera [7].

IItpm4 - M. mesonoto-pleuralis anterior [28 (50)].

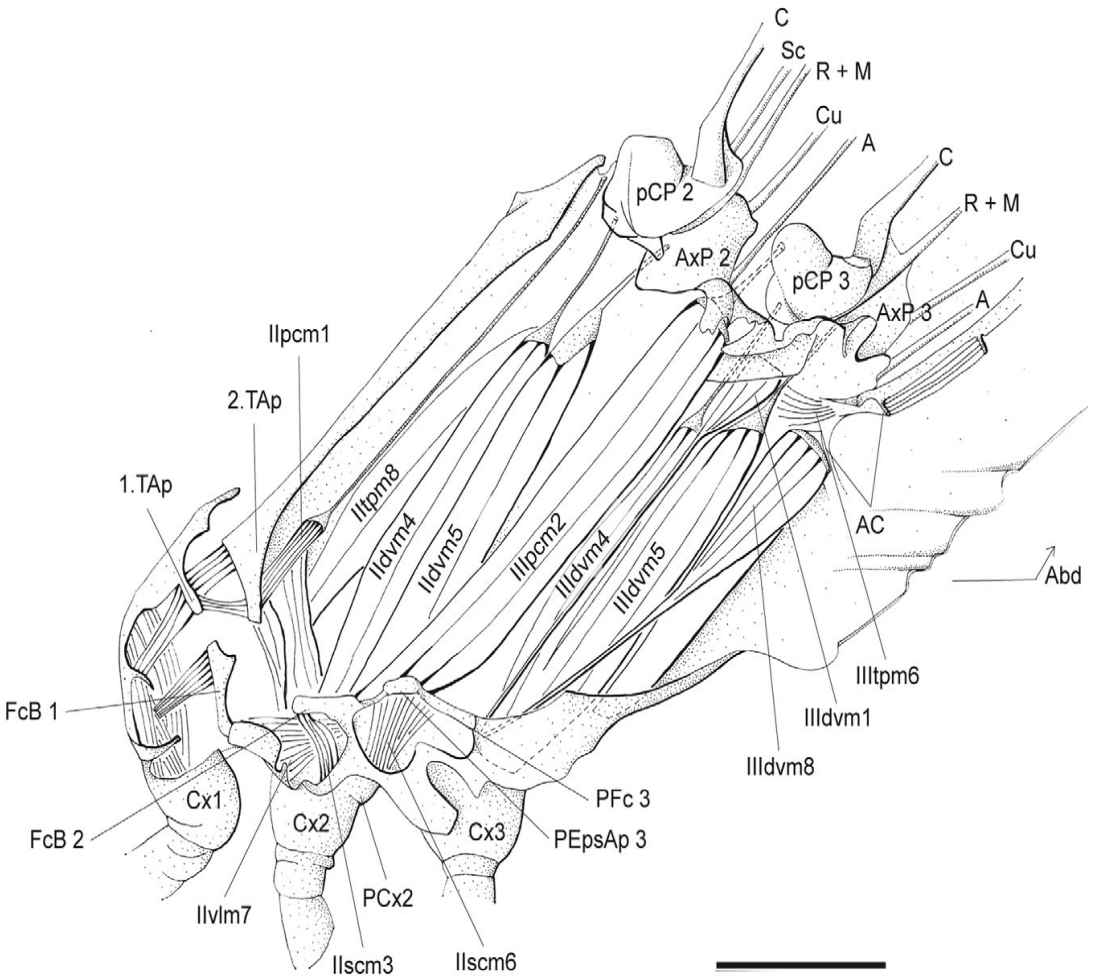

Figure 3. ***P. nymphula*****, thorax musculature, inner musculature removed, longitudinal cut, right.** Abbreviations: A – anal vein, Abd – abdominal segment, AC – antecosta, AxP – axillary plate, C – costa, Cu – cubitus, Cx – coxa, FcB – furcalbranch, M – media, pCP – proximale costal plate, PCx – precoxa, PEpsAp – preepisternal apodem, PFc – prefurca, R – radius, Sc – subcosta, TAp – tergal aphophyse.
doi:10.1371/journal.pone.0055787.g003

Origin: Pleural bar 2, close to the dorsal bifurcation.

P. nymphula (Fig. 1, 4).

Insertion: Median semi-detached scutal plate.

P.nymphula (Fig. 4), *C. splendens* (Fig. 11).

Characteristics: This muscle inserts at the lateral wall of the apodem of IIdvm3. It is an indirect tonic flight muscle [10].

IItpm2 - M. mesopleura-praealaris (new muscle).

Origin: Dorsal region of pleuralbar 2, dorsal of muscle IItpm9.

P. nymphula (Fig. 4, 5, 7A).

Insertion: Median semi-detached scutal plate, posterior of IIpcm1.

P.nymphula (Fig. 4, 5).

Characteristics: This muscle is strongly developed in *E. cyathigerum* and in *I. elegans*. It is missing in *C. splendens* and L. viridis. Its presence in *P. pennipes* could not be confirmed. This muscle was not described for Odonata so far. It is thin and elongate and runs almost parallel to muscle IIpcm1. It assumes a similar function as IIpcm1 and/or is reinforcing it.

IItpm9 - M. mesepimero-axillaris tertius [29/30 (51/52)].

Origin: With a short cap tendon at the posterior pleural process.

P. nymphula (Fig. 6B).

Insertion: In longitudinal axis at the ventral part of AxP2, precisely at the internal, caudal side of AxP2 next to the base of the anal vein.

P.nymphula (Fig. 6B, C).

Characteristics: IItpm4 and IItpm9 are located close together at AxP2, between the epifulcrum and the dorsal sclerite. Muscle IItpm4 is stronger and located more ventral; both have a cranial cap tendon. In *C. splendens* these muscles are distinctly separated from each other.

IItpm6 - M. mesonoto-pleuralis posterior [31 (53)].

Origin: Lateral on the mesoscutellum, close to the proximal edge of AxP2.

P.nymphula (Fig. 5, 8A).

Insertion: Pleural bar between mesepisternum and mesepimeron, close to the dorsal bifurcation.

P. nymphula (Fig. 4, 5, 8A), *C. splendens* (Fig. 11).

Characteristics: In Anisoptera this muscle is attached to the lateral wall of the apodem where also muscle IIdvm3 inserts [10].

IItpm8 - M. mesepimero-axillaris secundus [32 (54)].

Origin: Pleural bar between mesepimeron and katepisternum 2.

P. nymphula (Fig. 1, 4, 7A), *C. splendens* (Fig. 11).

Insertion: With a short tendon at the epifulcrum of AxP2.

P.nymphula (Fig. 4, 6D, 8A).

Characteristics: It is a broad and strong muscle with a dorsal cap tendon. This muscle is a direct depressor [10].

IItpm7 - M. mesanepisterno-axillaris [33 (55)].

Origin: Ventral of muscle IItpm9.

Table 1. Muscle homologies.

Homologies (Friedrich & Beutel (2008) and this study)		Asahina (1954)	
Name	**Abbr.**	**Name**	**No. (Metath.)**
Mesothorax			
M. mesanepisterno-trochantinalis	IIpcm1	Sternopleural (Sternobasalar)	21 (43)
M. mesobasalare-trochantinalis	IIpcm2	Sternopleural (Sternobasalar)	22 (44)
M. mesonoto-trochantinalis posterior	IIdvm3	Tergosternal (anterior tergosternal)	23 (46)
M. mesonoto-sternalis	IIdvm1	Tergosternal (anterior tergosternal)	23′ (46′)
M. prophragma-mesophragmalis	IIdlm1	Dorsal (lateral dorsal)	25 (45)
M. mesonoto-coxalis anterior	IIdvm4	Coxal (Coxobasalar)	26 (48)
M. mesonoto-coxalis posterior	IIdvm5	Coxal (Coxobasalar)	27 (49)
M. mesonoto-pleuralis anterior	IItpm4	Tergopleural	28 (50)
M. mesopleura-praealaris	IItpm2	–	–
M. mesepimero-axillaris tertius	IItpm9	Tergopleural (pleuro- RAP)	29/30 (51/52)
M. mesonoto-pleuralis posterior	IItpm6	Tergopleural (pleuro-RAP)	31 (53)
M. mesepimero-axillaris secundus	IItpm8	Tergopleural (pleurosubalar)	32 (54)
M. mesanepisterno-axillaris	IItpm7	Tergopleural (pleurosubalar)	33 (55)
M. mesepimero-subalaris	IItpm10	Tergopleural (pleurosubalar)	34 (56)
M. mesanepisterno-coxalis posterior	IIpcm4	Coxal (pleurocoxal)	36 (58)
M. mesofurca-coxalis medialis	IIscm3	Coxal (sternocoxal)	38 (61)
M. mesopleura-trochanteralis	IIpcm6	Trochanteral (Pleurotrochanteral)	39 (62)
M. mesofurca-trochanteralis	IIscm6	Trochanteral (Pleurotrochanteral)	40 (63)
M. profurca-mesofurcalis	Ivlm7	Ventral	41
Metathorax			
M. metanepisterno-trochantinalis	IIIpcm1	Sternopleural (Sternobasalar)	43
M. metabasalare-trochantinalis	IIIpcm2	Sternopleural (Sternobasalar)	44
M. mesophragma-metaphragmalis	IIIdlm1	Dorsal (lateral dorsal)	45
M. metanoto-phragmalis	IIIdlm2	Dorsal (lateral dorsal)	45′
M. metanoto-trochantinalis	IIIdvm3	Tergosternal (anterior tergosternal)	46
M. metanoto-sternalis	IIIdvm1	Tergosternal (anterior tergosternal)	46′
M. metanoto-coxalis anterior	IIIdvm4	Coxal (Coxobasalar)	48
M. metanoto-coxalis posterior	IIIdvm5	Coxal (Coxobasalar)	49
M. metanoto-pleuralis anterior	IIItpm4	Tergopleural	50
M. metapleura-praealaris	IIItpm2	–	–
M. metapimero-axillaris tertius	IIItpm9	Tergopleural (pleuro- RAP)	51/52
M. metanoto-pleuralis posterior	IIItpm6	Tergopleural (pleuro-RAP)	53
M. metepimero-axillaris secundus	IIItpm8	Tergopleural (pleurosubalar)	54
M. metanepisterno-axillaris	IIItpm7	Tergopleural (pleurosubalar)	55
M. metepimero-subalaris	IIItpm10	Tergopleural (pleurosubalar)	56
M. metanepisterno-coxalis posterior	IIIpcm4	Coxal (pleurocoxal)	58
M. metafurca-coxalis medialis	IIIscm3	Coxal (sternocoxal)	61
M. metapleura-trochanteralis	IIIpcm6	Trochanteral (Pleurotrochanteral)	62
M. metafurca-trochanteralis	IIIscm6	Trochanteral (Pleurotrochanteral)	63
Tendon	–	Ventral (Profurcoabdominal) Tendon	64
M. metaspina-abdominosternalis	IIIvlm3	Ventral (Profurcoabdominal)	66
M. metafurca-phragmalis	IIIdvm8	Tergosternal (posterior tergosternal)	67
M. mesofurca-abdominosternalis	IIIvlm2	Ventral	68

doi:10.1371/journal.pone.0055787.t001

P. nymphula (Fig. 1, 4, 6C), *C. splendens* (Fig. 11).
Insertion: With a short tendon at the postregion of AxP2. *P.nymphula* (Fig. 4).

Characteristics: This muscle has a cap tendon and runs similar to IItpm9, but in comparison it is distinctly smaller. It is a direct depressor muscle [10].

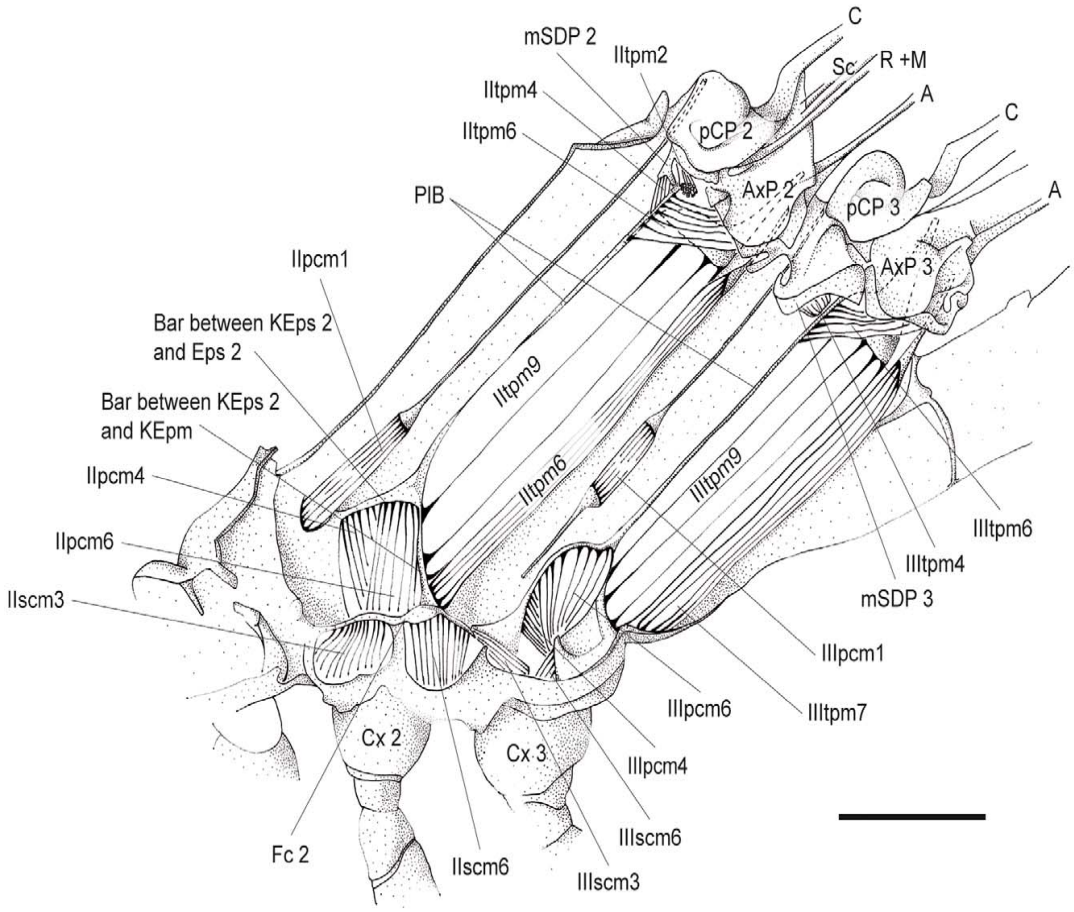

Figure 4. ***P. nymphula*****, lateral thorax musculature, longitudinal cut, right.** Abbreviations: A – anal vein, AxP – axillary plate, C – costa, Cx – coxa, Eps – episternum, KEpm – katepimerom, KEps – katepisternum, M – media, mSDP - mediane semi-detached scutal plate, pCP – proximale costal plate, PlB - pleuralbar, R – radius, Sc – subcosta.
doi:10.1371/journal.pone.0055787.g004

IItpm10 - M. mesepimero-subalaris [34 (56)].

Origin: In the middle at the pleural segmental border between meso- and metathorax.

P. nymphula (Fig. 1, 5, 6D, 8C).

Insertion: With a short tendon in the posterior region of AxP2, caudal of muscle IItpm6.

P. nymphula (Fig. 1, 5).

Characteristics: A short and thin muscle, with a dorsal, small cap tendon. The muscle is attached through resilin at the dorsal end [10].

IIpcm4 - M. mesanepisterno-coxalis posterior [36 (58)].

Origin: Lateral side of the anterior edge of the mesocoxa.

P. nymphula (Fig. 1, 4), *C. splendens* (Fig. 11).

Insertion: Pleural bar between mesepisternum and katepisternum 2.

P.nymphula (Fig. 1, 4), *C. splendens* (Fig. 11).

Characteristics: A broad and flat muscle, running very close to katepisternum 2.

IIscm3 - M. mesofurca-coxalis medialis [38 (61)].

Origin: Caudal at the basal side of the mesocoxa.

P. nymphula (Fig. 4).

Insertion: Ventral side of the furca branch 2.

P.nymphula (Fig. 7B).

IIpcm6 - M. mesopleura-trochanteralis [39 (62)].

Origin: Similar to muscle IIpcm4, at the lateral side of the mesocoxa.

P. nymphula (Fig. 1, 4), *C. splendens* (Fig. 11).

Insertion: Similar to muscle IIpcm4, at the pleural bar between mesepisternum 2 and katepisternum 2.

P. nymphula (Fig. 4), *C. splendens* (Fig. 11).

Characteristics: IIpcm4 and IIscm3 run in parallel with IIpcm4 being slightly more laterally.

IIscm6 - M. mesofurca-trochanteralis [40 (63)].

Origin: Latero-caudal at the base of the trochanter 2.

P. nymphula (Fig. 4), *C. splendens* (Fig. 11).

Insertion: At the proximal side of the prefurca 2.

P. nymphula (Fig. 7B), *C. splendens* (Fig. 11).

Characteristics: Lateral of muscle IIpcm4.

Ivlm7 - M. profurca-mesofurcalis [41].

Origin: Furca-branch 2.

P. nymphula (Fig. 2, 7B).

Insertion: Furca 1.

P. nymphula (Fig. 2, 7B).

42 Ventral (profurco-abdominal) M.

This structure has been described as a muscles [7], however, in the adult Zygoptera at this position only a tendon-like structure could be identified (cf. Asahina's 64 in metathorax).

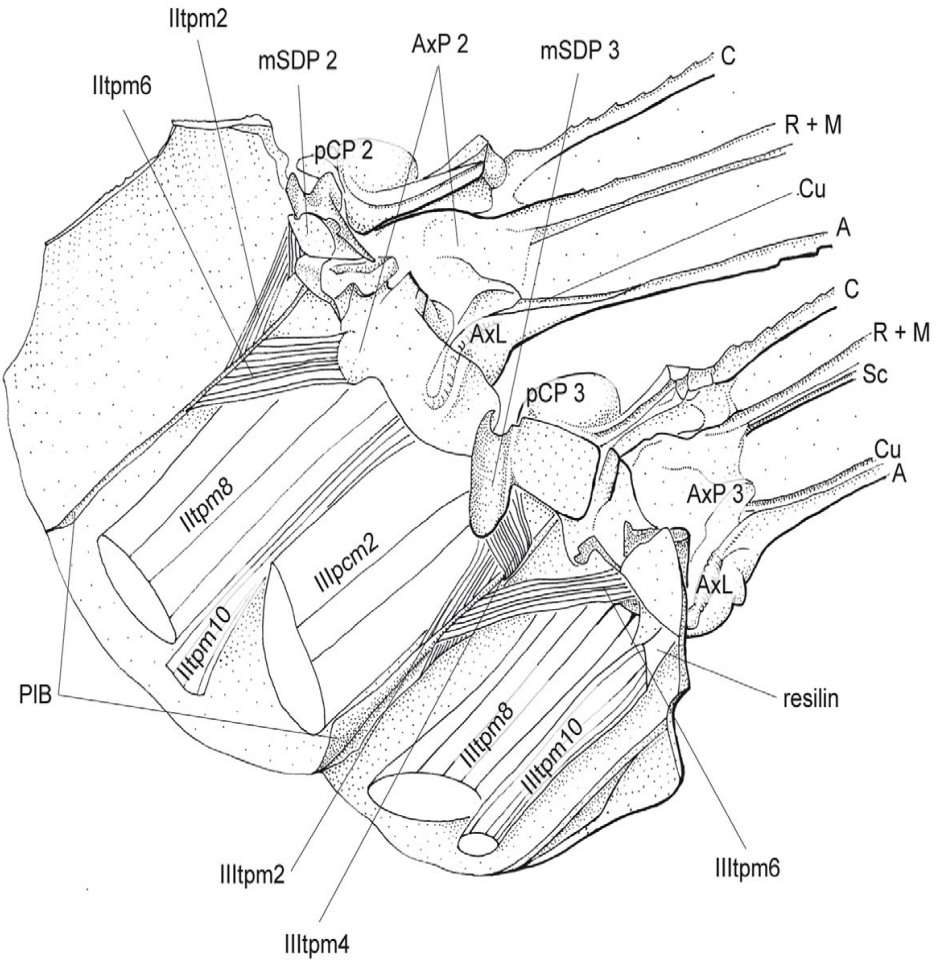

Figure 5. ***P. nymphula*****, detail of dorasl area of lateral flight musculature, longitudinal section, right.** Abbreviations: A – anal vein, AxL – axillary ligament, AxP – axillary plate, C – costa, Cu – cubitus, M – media, mSDP - mediane semi-detached scutal plate, pCP – proximale costal plate, PlB - pleuralbar, R – radius, Sc – subcosta.
doi:10.1371/journal.pone.0055787.g005

Musculature of the Metathorax

IIIpcm1 - M. metanepisterno-trochantinalis [43].

Origin: With a long tendon at the segmental border between epimeron 2 and episternum 3.

P. nymphula (Fig. 1, 4).

Insertion: With a long tendon at the membrane of proximal coxal plate three (pCP3).

P. nymphula (Fig. 1, 4).

Characteristics: A short muscles with cap tendons at both ends. These cap tendons are each attached to the cuticle through long tendons.

IIIpcm2 - M. metabasalare-trochantinalis [44].

Origin: Preepisternal apodem 3 [7].

P. nymphula (Fig. 2), *C. splendens* (Fig. 10, 12).

Insertion: At the edge of the pCP3.

P. nymphula (Fig. 3), *C. splendens* (Fig. 10).

Characteristics: The muscle has a dorsal cap tendon.

IIIdlm1 - M. mesophragma-metaphragmalis [45].

Origin: Proximal end of the tergal apophysis 4.

P. nymphula (Fig. 2), *C. splendens* (Fig. 10).

Insertion: Dorsal of the antecosta between abdomen and thorax.

P. nymphula (Fig. 2), *C. splendens* (Fig. 10).

Characteristics: In *P. latipes* the muscle has a flattend end, it is broader in Zygoptera than in Anisoptera [10].

IIIdlm2 - M. metanoto-phragmalis [45'].

Origin: Scutellum, close to the base of the tergal apophysis 4.

P. nymphula (Fig. 2, 8E),

Insertion: Proximal end of the tergal apophysis 4, dorso-lateral of muscle IIIdlm1.

P.nymphula (Fig. 8E), *C. splendens* (Fig. 9).

Characteristics: This muscle is only present in the metathorax of Zygoptera and *Epiophlebia*. In *Epiophlebia* it is distinctly thinner [7].

IIIdvm3 - M. metanoto-trochantinalis [46].

Origin: Broad at the postero-median region of the metascutum.

P.nymphula (Fig. 6E), *C. splendens* (Fig. 9).

Insertion: Cranial at the base of the coxa 3.

P. nymphula (Fig. 2), *C. splendens* (Fig. 9).

Characteristics: Very strong muscle.

IIIdvm1 - M. metanoto-sternalis [46'].

Origin: Lateral region of the metascutum, postero-median to muscle IIIdvm3.

P. nymphula (Fig. 3), *C. splendens* (Fig. 10).

Insertion: With a long tendon at the prefurca 3.

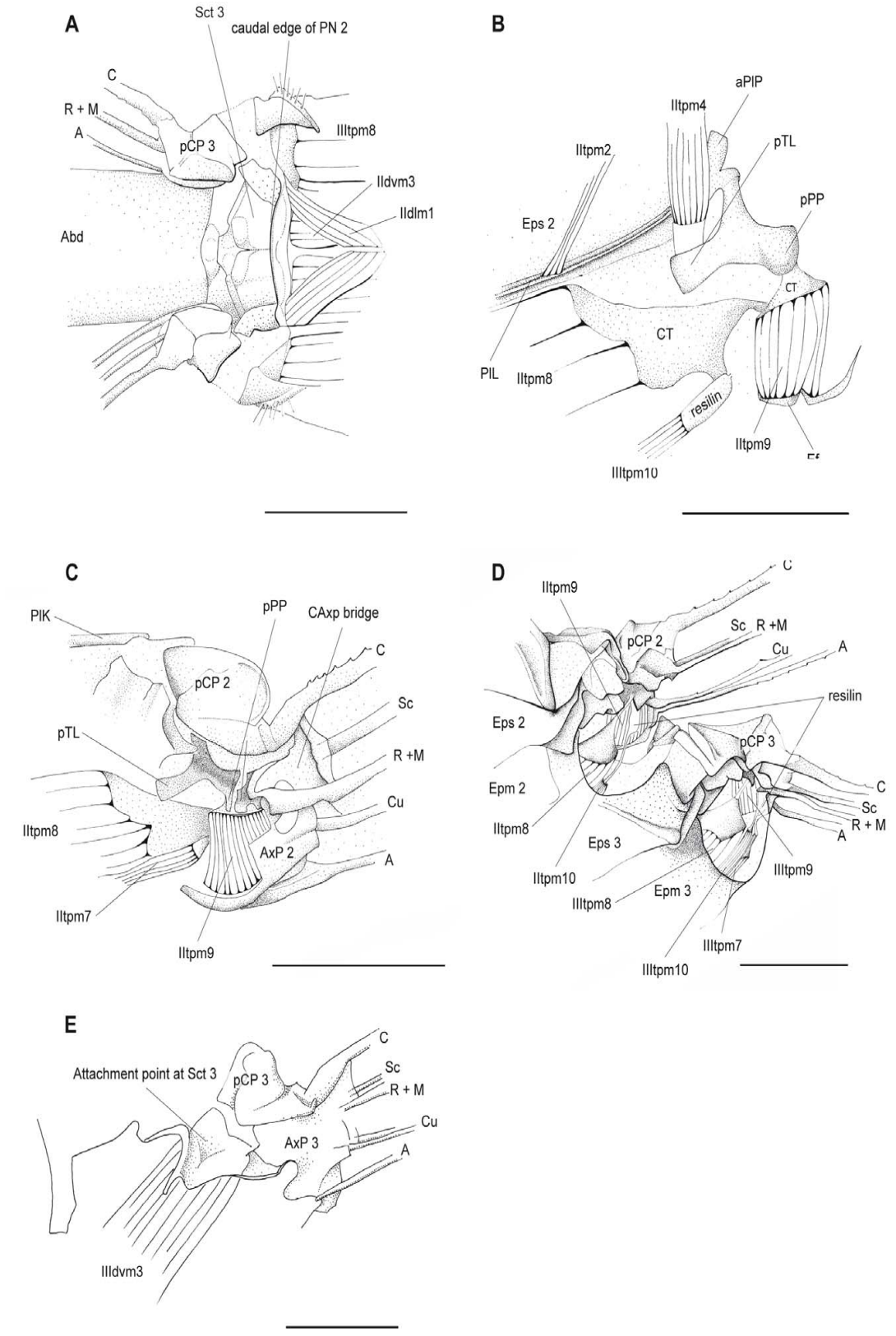

Figure 6. ***P. nymphula*****, thorax details.** A Points of origin of muscles IIdlm1, IIIdvm3 and IIItpm9. Dorsal view. B Detail of lateral flight muscles, longitudinal section, right. C Attachment points of muscles IItpm9, IItpm6 and IItpm8. Longitudinal section, right. D Detail of wing articulation area of the meso- and metathorax, left lateral view. E Attachment of muscle III dvm3. Longitudinal section, right. Abbreviations: A – anal vein, aPlP - anterior pleural process, AxP – axillary plate, C – costa, CAxP bridge - costa-axillary plate bridge, CT – cap tendon, Cu – cubitus, Epm – epimeron, Eps – episternum, M – media, pCP - proximale costal plate, PIL - pleuralbar, PIK – pleuralkeel, PN – postnotum, pPP – posterior pleural process, pTL - posterior tergal levler, R – radius, Sc – subcosta, Sct – scutum.
doi:10.1371/journal.pone.0055787.g006

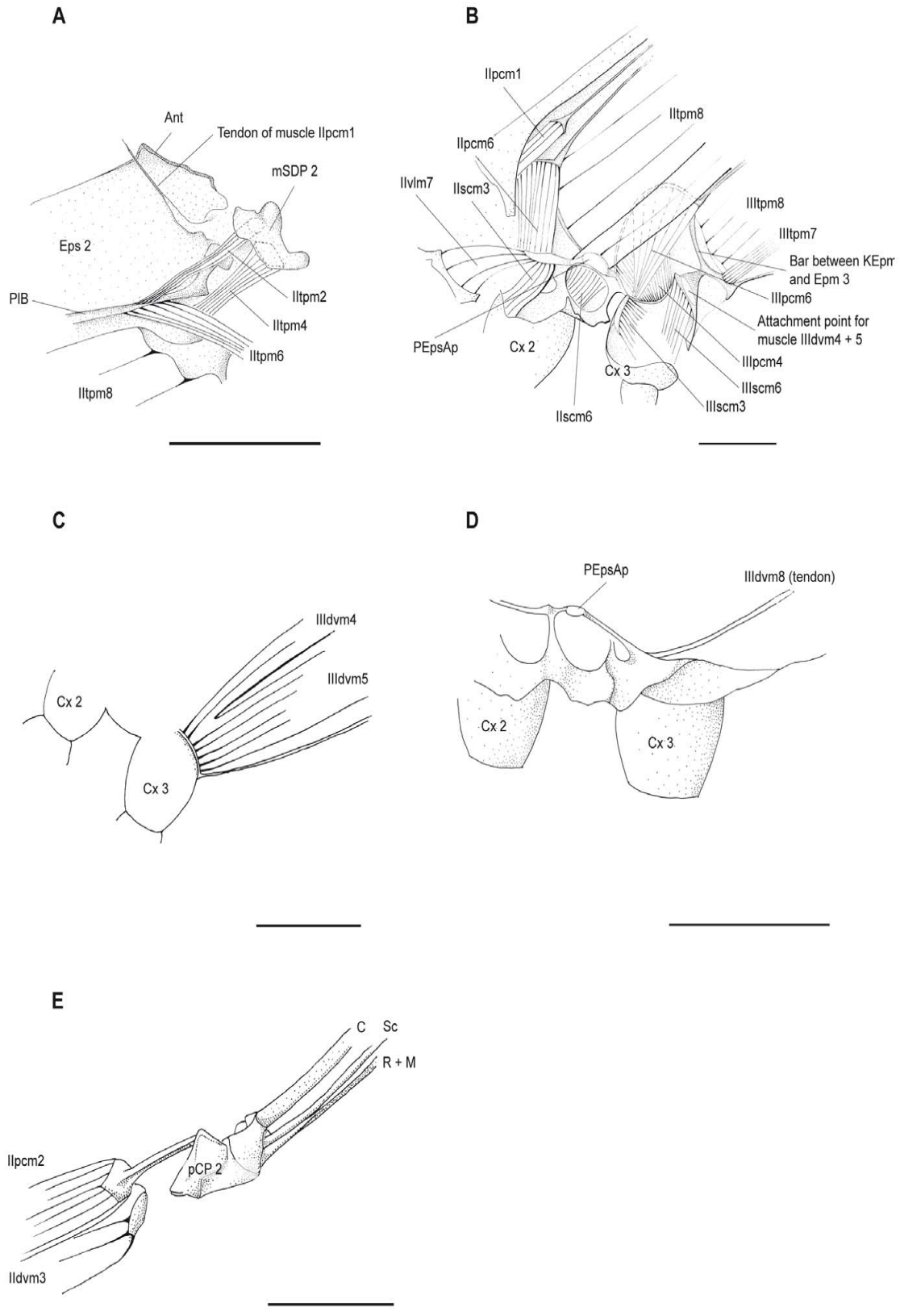

Figure 7. *P. nymphula*, details of muscle attachements. A–D Longitudinal section, right. A Attachments point of muscles IItpm4, IIItpm4, IIItpm6 and IItpm8. B Ventral thorax musculature. C Points of origin of muscles IIIdvm4 and IIdvm5. D Point of origin of muscle IIIdvm8. E Detail of attachment points of muscles IIpcm2, IIdvm3. Dorsal view, right. Abbreviations: Ant – antealar plate, C – costa, Cx – coxa, Epm – epimeron, Eps – episternum, KEpm – katepimerom, M – media, mSDP - mediane semi-detached scutal plate, PEpsAp – preepisternal apodem, pCP - proximale costal plate, PIB – pleuralbar, R – radius, Sc – subcosta.
doi:10.1371/journal.pone.0055787.g007

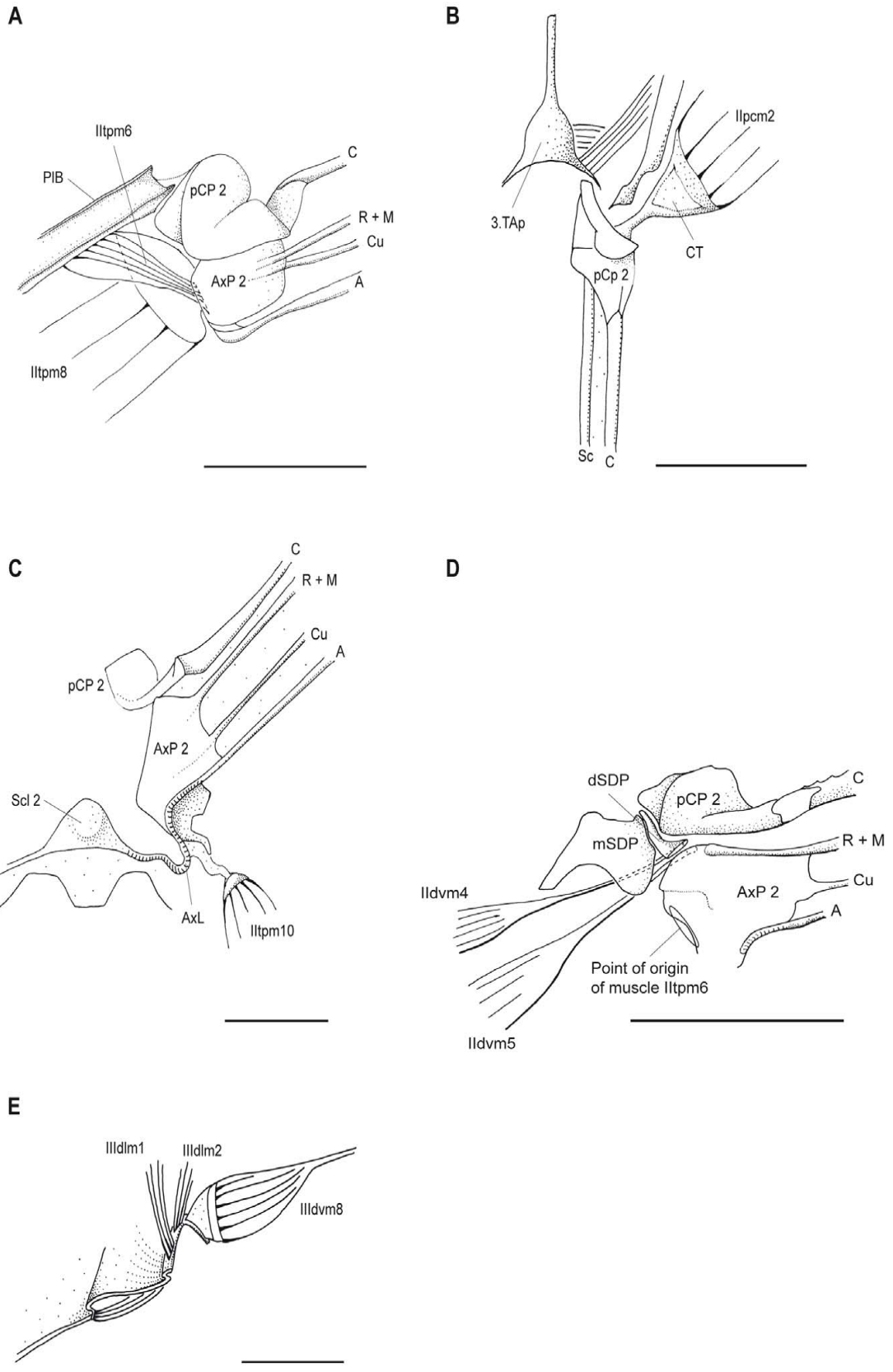

Figure 8. ***P. nymphula*****, details of muscle attachment points.** A, D and E Longitudinal section, right. B and C Dorsal view, right. A Point of origin of muscle IItpm6. B Attachment point of muscle IIpcm2. C Attachment point of muscle IItpm10. D Points of origin of muscles IIdvm4, IIdvm5 and IItpm6. E Points of origin of muscles IIIdlm1, IIIdlm2 and IIIdvm8. Abbreviations: A – anal vein, AxL – axillary ligament, AxP – axillary plate, C – costa, CT – cap tendon, Cu – cubitus, dSDP - distal semi-detached scutal plate, M – media, mSDP - mediane semi-detached scutal plate, pCP – proximale costal plate, PlB – pleuralbar, R – radius, Sc – subcosta, Scl – scutellum, TAp – tergal aphophyse.
doi:10.1371/journal.pone.0055787.g008

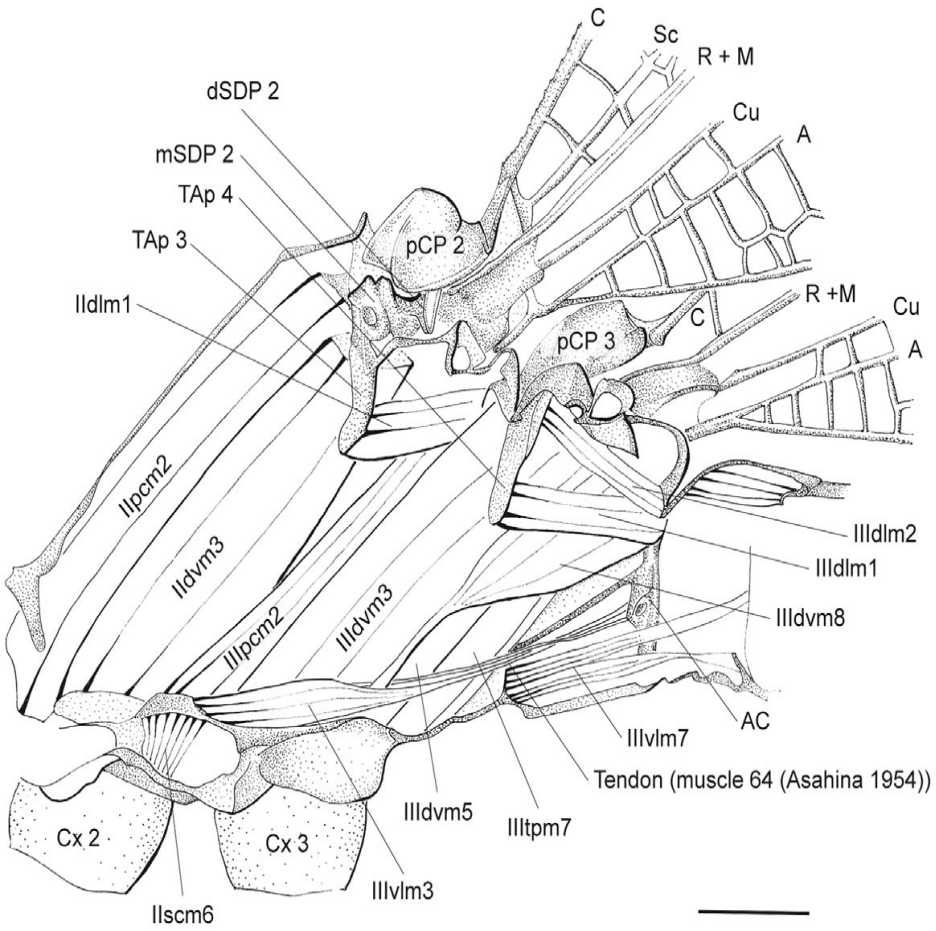

Figure 9. ***C. splendens*****, innermost layer of thorax musculature. Longitudinal cut, right.** Abbreviations: A – anal vein, AC – antecosta, C – costa, Cu – cubitus, Cx – coxa, dSDP - distal semi-detached scutal plate, M – media, mSDP - mediane semi-detached scutal plate, pCP – proximale costal plate, R – radius, Sc – subcosta, TAp – tergal aphophyse.
doi:10.1371/journal.pone.0055787.g009

P. nymphula (Fig. 3), *C. splendens* (Fig. 10).

Characteristics: This muscle corresponds to muscle IIdvm1 of the mesothorax, but is distinctly stronger. The presence in *P. pennipes* could not be confirmed. In *C. spelndens* the muscle is thin and elongate, whereas, in *E. elegans* it is quite small. It is missing in *Epiophlebia* [7].

IIIdvm4 - M. metanoto-coxalis anterior [48].

Origin: With a tendon caudal of the semi-detached scutal plate, below the anterior area of pCP3.

C. splendens (Fig. 11).

Insertion: Mesocoxal disk at the basal edge of the coxa 3.

P. nymphula (Fig. 7B, C), *C. splendens* (Fig. 11).

Characteristics: See muscle IIIdvm5.

IIIdvm5 - M. metanoto-coxalis posterior [49].

Origin: With a tendon at axillary plate three (AxP3), proximo-caudal from muscle IIIdvm4 at the origin of the radius-media vein.

C. splendens (Fig. 11).

Insertion: Mesocoxal disk at the basal edge of the coxa 3, posterior to muscle IIIdvm4.

P. nymphula (Fig. 7B,C), *C. splendens* (Fig. 11).

Characteristics: IIIdvm4 and IIIdvm5 share the same point of origin at the coxa 3. Each muscle inserts via a long tendon. They have dorsal cap tendons and serve as direct flight muscles.

IIItpm4 - M. metanoto-pleuralis anterior [50].

Origin: Pleural bar 3, close to the dorsal bifurcation.

P. nymphula (Fig. 4, 5), *C. splendens* (Fig. 9, 10).

Insertion: Median semi-detached scutal plate.

P.nymphula (Fig. 5).

Characteristics: This muscle inserts at the lateral wall of the apodem where also muscle IIIdvm3 is attached. It is an indirect tonic flight muscle [10]. The muscle is stronger than its relative in the mesothorax.

IIItpm2 - M. metapleura-praealaris (new muscle).

Origin: Dorsal region of the pleural bar between episternum 3 and epimeron 3, dorsal of muscle IIItpm9.

P. nymphula (Fig. 5).

Insertion: Median semi-detached scutal plate.

Characteristics: This muscle is strongly developed in *E. cyathigerum* and in *I. elegans* but it is missing in *C. splendens* and *L. viridis*. Its presence in *P. pennipes* could not be confirmed. It is thin and elongate, runs almost parallel to IIpcm1 and was not described in Odonata so far. It assumes a similar function as muscle IIpcm1 and/or is reinforcing it.

IIItpm9 - M. metapimero-axillaris tertius [51/52].

Origin: A short cap tendon at the posterior pleural process.

P. nymphula (Fig. 6D), *C. splendens* (Fig. 9, 10).

Insertion: In longitudinal axis at the ventral part of AxP 3, precisely at the internal, caudal side of AxP3 next to the base of the anal vein.

P. nymphula (Fig. 6D), *C. splendens* (Fig. 9).

Characteristics: Both muscles are located close together at AxP 3, between the epifulcrum and the dorsal sclerite. Muscle IIItpm4

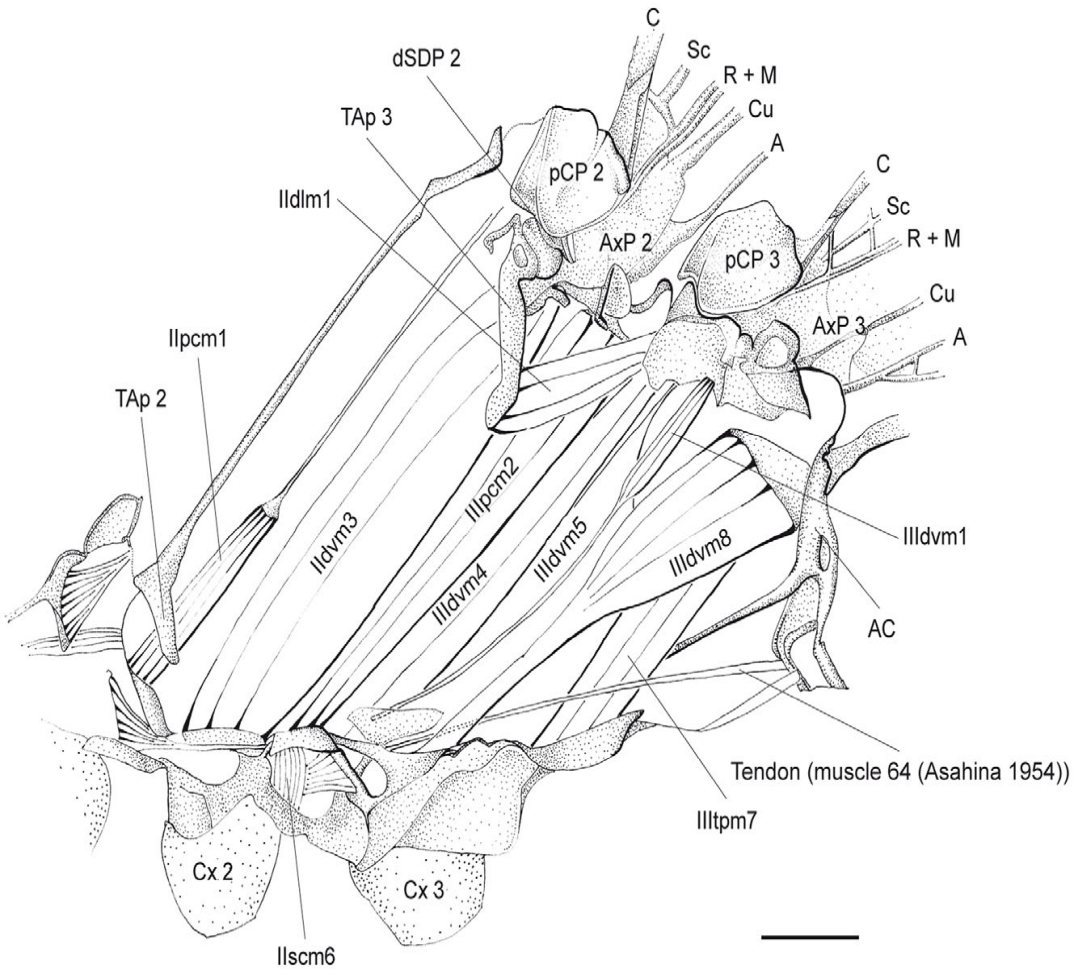

Figure 10. ***C. splendens*****, thorax musculature. Inner musculature removed, longitudinal cut, right.** Abbreviations: A – anal vein, AC – antecosta, AxP – axillary plate, C – costa, Cu – cubitus, Cx – coxa, dSDP - distal semi-detached scutal plate, M – media, pCP – proximale costal plate, R – radius, Sc – subcosta, TAp – tergal aphophyse.
doi:10.1371/journal.pone.0055787.g010

is stronger and located more ventral; both have a cranial cap tendon. In *C. splendens* these muscles are distinctly separated from each other.

IIItpm6 - M. metanoto-pleuralis posterior [53].

Origin: Proximal edge of AxP 3.

P. nymphula (Fig. 4), *C. splendens* (Fig. 11).

Insertion: Dorsally on the pleural bar 3.

P. nymphula (Fig. 4, 5), *C. splendens* (Fig. 11).

Characteristics: The muscle is stronger than its relative in the mesothorax.

IIItpm8 - M. metepimero-axillaris secundus [54].

Origin: Bar between epimeron 3 and katepisternum 3.

P. nymphula (Fig. 1, 4, 6A, D, 7B), *C. splendens* (Fig. 12).

Insertion: Through a tendon at the epifulcrum of the AxP 3, at the elongation of the cubitus.

C. splendens (Fig. 12).

Characteristics: It is a broad and flat muscle, with a dorsal cap tendon.

IIItpm7 - M. metanepisterno-axillaris [55].

Origin: Bar between epimeron 3 and katepisternum 3.

P. nymphula (Fig. 1, 4, 7B), *C. splendens* (Fig. 11).

Insertion: With a short tendon at the posterior region of the AxP 3, posterior to muscle IIItpm9.

P. nymphula (Fig. 6D), *C. splendens* (Fig. 11).

Characteristics: The muscle has a dorsal cap tendon.

IIItpm10 - M. metepimero-subalaris [56].

Origin: Bar between epimeron 3 and poststernum 3 [7].

P.nymphula (Fig. 1), *C. splendens* (Fig. 11).

Insertion: With a short tendon at the posterior region of the AxP 3.

P. nymphula (Fig. 5, 6B, D).

Characteristics: It is a short and thin muscle, which is attached through resilin [10] at the dorsal end (cf. IItpm8).

IIIpcm4 - M. metanepisterno-coxalis posterior [58].

Origin: Bar between the katepisternum 3 and episternum 3.

P. nymphula (Fig. 7B).

Insertion: Lateral of the posterior edge of the coxa 3.

P. nymphula (Fig. 1, 7B).

IIIscm3 - M. metafurca-coxalis medialis [61].

Origin: Furca 3.

P. nymphula (Fig. 7B).

Insertion: Antero-lateral edge of the coxa 3.

P. nymphula (Fig. 2, 4, 7B).

IIIpcm6 - M. metapleura-trochanteralis [62].

Origin: Bar between katepisternum 3 and episternum 3, median of the muscle IIIpcm4.

P. nymphula (Fig. 1, 7B), *C. splendens* (Fig. 12).

Insertion: Antero-lateral of the coxa 3.

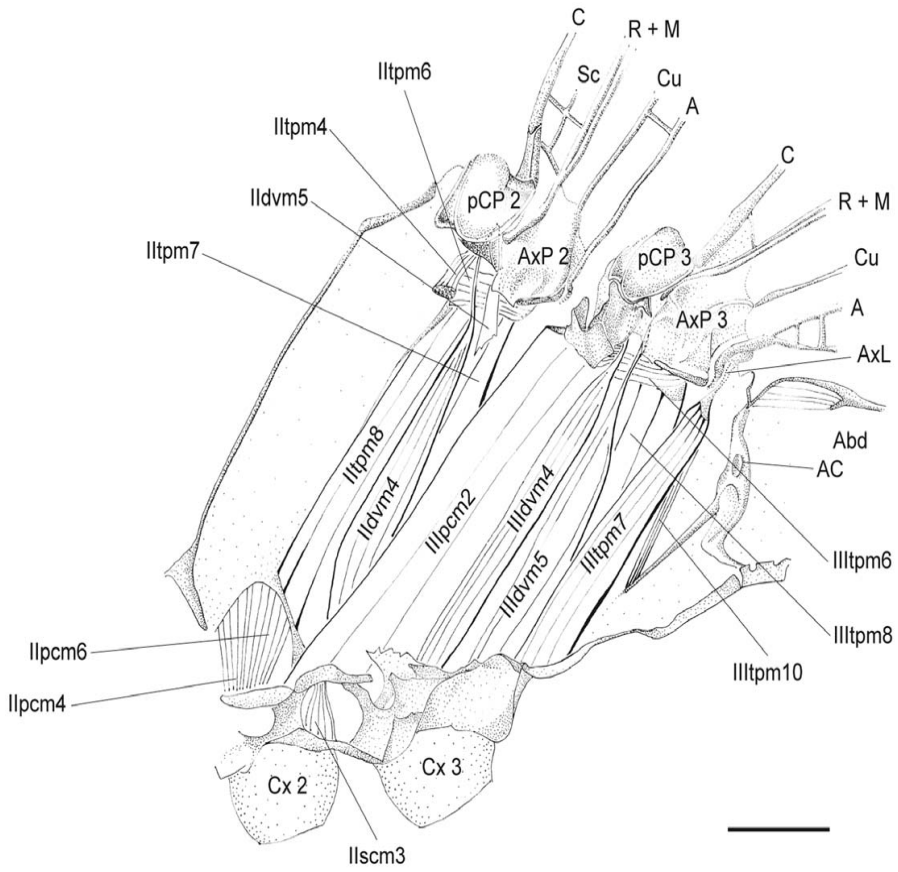

Figure 11. ***C. splendens*****, thorax musculature, longitudinal cut, right.** Abbreviations: A – anal vein, Abd – abdominal segment, AC – antecosta, AxL – axillary ligament, AxP – axillary plate, C – costa, Cu – cubitus, Cx – coxa, M – media, pCP – proximale costal plate, R – radius, Sc – subcosta. doi:10.1371/journal.pone.0055787.g011

P. nymphula (Fig. 1, 7B), *C. splendens* (Fig. 12).

Characteristics: In *Epiophlebia* this muscle inserts at the base of the trochanter 3 [7].

IIIscm6 - M. metafurca-trochanteralis [63].

Origin: Proximal at the furca branch 3.

P. nymphula (Fig. 1, 7B).

Insertion: Base of the trochanter 3.

P. nymphula (Fig. 1, 2, 4, 7B).

64 Ventral (Profurcoabdominal) **Tendon** (cf. 42 in mesothorax).

Origin: Furca 1.

C. splendens (Fig. 11).

Insertion: Bar between epimeron 3 and 1. abdominal sternite, lateral of muscle IIIvlm3.

P. nymphula (Fig. 1, 4), *C. splendens* (Fig. 10).

Characteristics: This structure has been described as a muscle [7]. In the Zygoptera investigated it shows no muscle fibers.

IIIvlm3 - M. metaspina-abdominosternalis [66].

Origin: Caudal of the poststernum 3.

P. nymphula (Fig. 2), *C. splendens* (Fig. 9).

Insertion: Bar between 1. and 2. abdominal sternite.

P. nymphula (Fig. 2, 8E), *C. splendens* (Fig. 9).

Characteristics: In *C. splendens* this muscle is distinctly flatened caudally.

IIIdvm8 - M. metanoto-phragmalis [67].

Origin: With a long tendon at the anterior edge of the furca invagination [7].

P. nymphula (Fig. 3, 7D), *C. splendens* (Fig. 11), P. latipes (Fig. 8D).

Insertion: Posterior edge of the 1. abdominal tergite.

P. nymphula (Fig. 3), *C. splendens* (Fig. 10).

Characteristics: This muscle has a dorsal cap tendon that inserts directly at the antecosta. The muscle is broader and shorter than in Anisoptera [10]. The cap tendon in *I. elegans* inserts ventral of muscle IIIscm6. In *L. viridis* the cap tendon is weakly developed.

IIIvlm2 - M. mesofurca-abdominosternalis [68].

Origin: Proximal side of the prefurca (at the anterior part of the furca invagination [7] or at the intersegmental apophysis of the pleural sternite [23]).

P. nymphula (Fig. 2), *C. splendens* (Fig. 9).

Insertion: With a long tendon at the bar between epimeron 3 and 1. abdominal sternite.

P.nymphula (Fig. 2), *C. splendens* (Fig. 9).

Characteristics: This muscle has a distinctly elongated tendon. In *C. splendens* it is very thin but the sclerotisations of the cap tendon and of the tergal sclerite are stronger than in the other species investigated. The cap tendons in *L. viridis* are either sparsely developed (IIpcm1, IIpcm2, IIdvm3, IIItpm4, IIIdvm3) or abent (IIdvm4, IIdvm5, IIIdvm4, IIIdvm5).

Discussion

Asahina [7] listed 51 thorax muscles for Odonata in general and 42 muscles for adult Zygoptera. Of these muscles 19 belong to the mesothorax with Zygoptera lacking muscles 35 and 37. The remaining 23 muscles belong to the metathorax where Zygoptera do not have muscle 47, 57 and 60. In our study the 42 muscle of Asahina [7] could be confirmed. Additionally, four muscles (IIpcm2, IItpm2, IIIpcm2, IIItpm2) were found that were

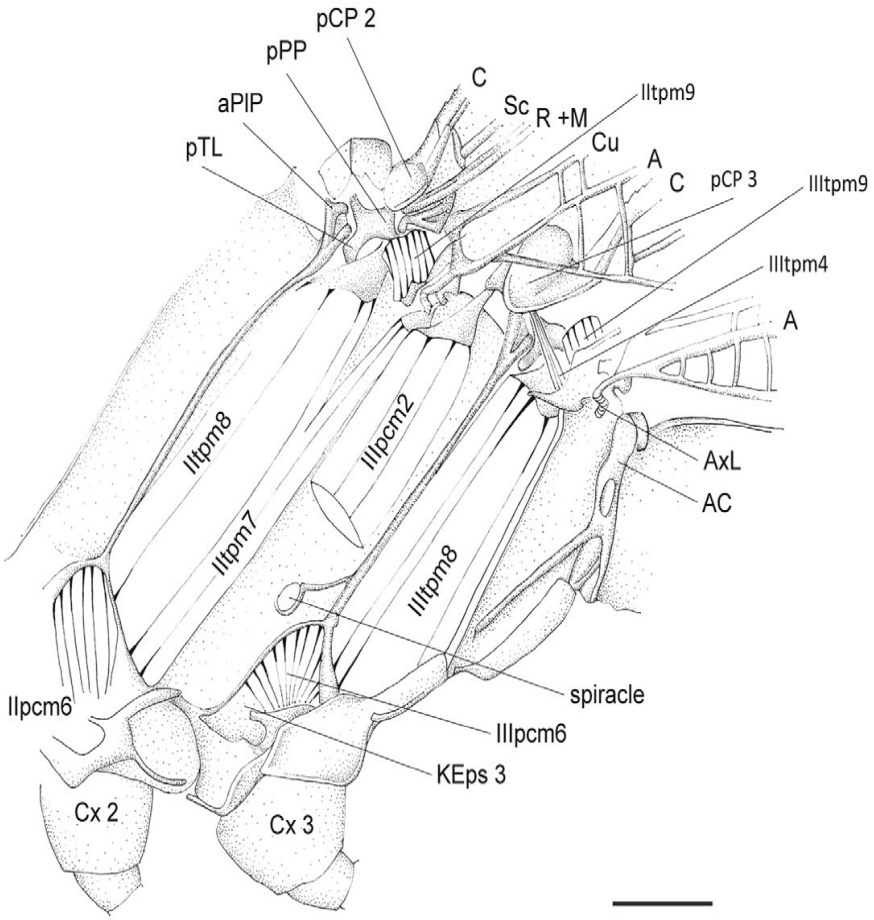

Figure 12. *C.splendens*, lateral thorax musculature, longitudinal cut, right. Abbreviations: A – anal vein, AC – antecosta, aPlP - anterior pleural process, AxL – axillary ligament, C – costa, Cu – cubitus, Cx – coxa, KEps – katepisternum, M – media, pCP – proximale costal plate, pPP – posterior pleural process, pTL - posterior tergal levler, R – radius, Sc – subcosta.
doi:10.1371/journal.pone.0055787.g012

previously not known for the Odonata (cf. [5,7,10,23]). IIpcm2 and IIIpcm2 are present in all species studied, with the exception of *L. viridis* and *C. splendens*. In *P. pennipes* the condition is uncertain because of insufficient data.

The short and slender pleuro tergal muscles IItpm2 and IIItpm2 are run from the dorsal part of the pleural bar to the median semi-detached scutal plate (Fig. 7A). They have positions and directions similar to IItpm4 and IIItpm4. Therfore, we assume a similar or reinforcing function (cf. [10]).

A couple of observed origin and insertion points differ from Asahina's [7] descriptions. For example, IIdlm1 inserts at the anterior edge of the postnotum 2, not at the lateral side of the scutum 3 [7]. The muscles IIdlm1, IIIdlm1 and IIIdlm2 have been identified as indirect flight muscles [24]. They originate at the tergal apophysis and were previously homologized with dorsal longitudinal muscles of the neopteran pterothorax [10]. In the ground pattern of the Neoptera the longitudinal muscles run between the phragmata [3]. The point of insertion of muscle IIdlm1 at the caudal edge of the postnotum, i.e. at the caudal end of the second thorax segment, is equivalent to the position of the phragma in Neoptera, which supports the homologization proposed.

In *C. splendens* IIdvm4 and IIdvm5 originate at the distal base of the mesocoxa (cf. [7]). In the other seven species investigated, these muscles originate rather cranial at the anterior part of the mesocoxa. Since *Mnais strigata*, which was studied by Asahina [7] and *C. spledens* both belong to Calopterygidae, the translocation of the point of origin may well be an apomorphy of this group.

The points of origin of the corresponding metathorax muscles IIIdvm4 and IIIdvm5 differ from previous descriptions [7] in all species investigated. They are located caudal not distal of the base of the metacoxa.

Further more, IIdvm4 has been described as attaching to the inner caudal angle of the costal plate 2. In the Zygoptera investigated, IIdvm4, like its metathoracic homolog IIIdvm4, is attached to the lateral side of the semi-detached scutal plate. The muscles do not attach at the wing articulation, rather at a tergal sclerite. Therefore, they have to be characterized as indirect not as direct flight muscles [10]. This also applies to the strong indirect lifter IIdvm3 (and IIIdvm3), which is a main flight muscle and is also attached to the tergum.

Similarly, the pleuro-tergal muscles IItpm4, IIItpm4, IItpm2, IIItpm2, IItpm9, IIItpm9, IItpm6, IIItpm6 are all indirect flight muscles in the morphological sense, because they all insert on pleural or tergal sclerites.

The remaining muscles (IIpcm1, IIIpcm1, IIpcm2, IIIpcm2, IIdvm5, IIIdvm5, IItpm8, IIItpm8, IItpm7, IIItpm7, IItpm10, IIItpm10) are direct flight muscles since they are directly connected via tendons to the costal plate or to the axillary plate.

Consequently, the flight musculature of the Zygoptera consists of direct and historically indirect flight muscles. However, as far as

the functions of the dorso-ventrally arranged flight muscles are concerned, all are now acting as direct muscles.

The conspicuously long tendons (e.g. IIpcm1, IIIpcm1) are characteristic for the Zygoptera.

Homology of the Musculature of the Pterothorax in Zygoptera and Neoptera

Already in the descriptive part of this work we used the muscle nomenclature suggested by Friedrich and Beutel [18] for a generalized neopteran thorax. In the following the homologization of the flight musculature of Zygoptera with that of Neoptera is explained further (cf. Table 1, S1).

Dorsolongitudinal musculature (dlm). The tergal apophyses are intersegmental invaginations and therefore not homologous to the primary diaphragms of Neoptera [24], but presumably to the pseudo phragmata of other insects [25]. The zygopteran muscles IIdlm1, IIIdlm1 and IIIdlm2 originate at the tergal apophysis and their homology with the dorsolongitudinal musculature of Neoptera appears to be unequivocal [17].

Dorsoventral musculature (dvm). The points of origin and insertion of the zygopteran dorsoventral muscles are usually shifted to some degree in comparision to Neoptera. The reasons for this are not so much functional modifications, but drastic changes in shape and size of the notum of Odonata in coparision to that of other Pterygota. Nevertheless, the functions of these muscles as elevators of the wings are preserved. Their positions in the thorax together with the relationships to other muscles allow for a well-supported homologization. The muscles IIdvm1 and IIdvm3, IIdvm4, IIdvm5 could be identified in the odonatan thorax.

Ventral musculature (vlm). The ventral muscle system in the Zygoptera appears to be highly simplified. We could identify one unequivocal ventral longitudinal muscle only: Ivlm7 is identical in its origin and insertion to its neopteran relative [18]. It seems not to be present in the Anisoptera but was also found in the Ephemeroptera [17].

Tergopleural musculature (tpm). The muscles IItpm6 and IItpm2 originate dorsally at the pleural bar. Muscle IItpm6 inserts below the proximal region of the axillary plate. In Neoptera IItpm6 inserts on the 3. axillary. The proximal area of the odonatan axillary plate has been homologized with the 3. axillary of Neoptera [14], which supports our identification of this muscle.

IItpm2 inserts on the median semi-detached scutal plate. Therefore, a homology with either the neopteran IItpm2 or IItpm4 seems to be possible.

An identification of this muscle as IItpm4 could be excluded, because in Neoptera IItpm4 inserts on the 1. axillary [18], which in Odonata corresponds to the anterior-proximal area of the axillary plate [14]. Since IItpm2 inserts on the subtegula or on the prealare sclerite in Neoptera, which correspond to the odonatan scutal plate, our homologization appears to be most probable.

The points of origin of IItpm9 and IIItpm9 at the pleural processes of their segments as well as the points of insertion on the axillary plates (homologous region see above) correspond well to the situation in the Neoptera and also in the Ephemeroptera [17].

Due to the virtually identical points of origin and insertion in the Neoptera [18] as well as in the Odonata the homologization of the metathoracic muscles IIItpm4 and IIItpm6 appears to be unequivocal.

Pleuro-coxal musculature (pcm). The zygopteran muscles IIpcm1 and IIIpcm1 originate at the preepisternum of the corresponding segments at the anterior edge of the pCP. Due to the ventro-dorsal expansion of the pleura in Odonata, this sclerite is directed nearly ventrally. Therefore, the orientations of the muscles in the thorax differ from there relatives in the Neoptera. However, the points of origin and insertion together with the relation to other muscles support the homologization.

The zygopteran muscles IIpcm4, IIIpcm4, IIpcm6 and IIIpcm6 show the same points of origin and insertion as their neopteran counterparts. Together with functional considerations this supports the suggested homologization. Nevertheless, there is some variation in the points of insertion of IIpcm6 and IIIpcm6. In Zygoptera they insert on the trochanter of the corresponding segments, very close the insertion of IIpcm4 or IIIpcm4, respectively. In Anisoptera and in *Epiophlebia* these insertions are shifted to some degree [7].

In summary, our comparative investigation of the flight musculature of the Odonata shows that homologization with the flight musculature of Neoptera in most cases is realtively straightforward. Due to the significant modifications of the skeleton of the odonatan pterothorax many points of origin shifted in variing degrees. However, the general positions and orientations of the muscles are still persistent. It also became clear that the flight musculature of Zygoptera and of Odonata in general is composed of direct as well as indirect muscles as it is the case in the Neoptera. Those muscles that historically are indirect flight muscles work as direct flight muscles in the Odonata due to the modifications in their skelettal system, especially in the notal sclerites. With a well-supported homologization of the flight muscles between the Zygoptera (and consequently Odonata) and the Neoptera, this character system now can also be used to expand datasets for the analysis of phylogenetic relationships of all pterygote insects.

Materials and Methods

Odonata: Zygoptera.
Coenagrionidae.

- *Pyrrhosoma nymphula* (Sulzer, 1776): Billingshäuser Schlucht, Göttingen, Germany.
- *Coenagrion puella* (Linnaeus, 1758): Billingshäuser Schlucht, Göttingen, Germany.
- *Enallagma cyathigerum* (Charpentier, 1940): Billingshäuser Schlucht, Göttingen, Germany.
- *Ischnura elegans* (Vander Linden, 1820): Billingshäuser Schlucht, Göttingen, Germany.

Calopterygidae.

- *Calopteryx splendens* (Harris, 1782): Billingshäuser Schlucht, Göttingen, Germany and Villemur sur Tarn, France.

Platycnemidae.

- *Platycnemis latipes* (Rambur, 1842): Barsac, France.
- *Platycnemis pennipes* (Pallas, 1771): Barsac, France and Villemur sur Tarn, France.

Lestidae.

- *Lestes viridis* (Vander Linden, 1825): Barsac, France.

All regulations concerning the protection of free-living species were followed.

All necessary permits were obtained for collecting Odonata at the Billingshäuser Schlucht, Göttingen, Germany (permission granted by "Untere Naturschutzbehörde" file reference AZ.67.2.5 Wei). For collecting Damselflies in France, no specific permits are required. The locations where the damselflies were

collected are not privately owned or protected in any way. No endangered or especially protected species were collected.

The specimens were collected into 80% EtOH. Subsequently, they were fixed in Dubosq-Brasil fixative [26] and stored in 80% EtOH.

Specimens were studied, prepared and drawn with the help of a stereomicroscope (Zeiss Stemi SV11) with a camera lucida.

Synchrotron radiation micro computed tomography (SRμCT) was applied in order to generate data for three-dimensional reconstruction of the structures of interest. Prior to scanning, the samples were critical point dried (Balzer CPD030). The SRμCT data were generated at the Swiss Light Source (SLS) in Villigen (Switzerland), at the beamline TOMCAT, (Proposal no. 20080794 and Proposal no. 20100088, ThH) as well as at the Deutsches Elektronen Synchrotron (DESY) in Hamburg, Germany, (Proposal no. I-20090102, SB).

Three-dimensional reconstructions (processing and visualization) of the data were prepared with Amira® 5.2. (Visage Imaging, Richmond, Australia). All images were subsequently processed with Photoshop CS3 (Adobe System Inc., San José, USA).

Supporting Information

Table S1 Homologisation of thoracic muscle nomenclatures used by several authors. - absent/? uncertain. (XLSX)

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Author Contributions

Conceived and designed the experiments: SB TH. Performed the experiments: SB CG TH. Analyzed the data: SB CG TH. Wrote the paper: SB TH.

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Tab. S1: Homologisation of thoracic muscle nomenclatures used by several authors

- absent / ? uncertain

Friedrich & Beutel (2008)	this study	Asahina (1954)	Willkommen (2008)	Wittig (1955)	Matsuda (1970)
Mesothorax					
IIdlm1	x	25	MT.m	II dlm 35	t 14
IIdlm2	-	-	S.LPNm	II dlm 36, II dlm 37	t12, t13
IIdlm3	-	-	S.Esm	-	t-p 5, t-p 6
IIdvm1	x	23'	S.CmA	II dvm 40	t-ti 1, t-ti 2
IIdvm2	-	-	S.CmA	II dvm 41	t-ti 3
IIdvm3	x	23	-	-	t-cx 5
IIdvm4	x	26	PSL.Cm, S.CmP	II dvm 43	t-cx 6, t-cx 7
IIdvm5	x	27	SA.Cm, SA.Fm	II dvm 43	t-cx 8
IIdvm6	-	-	S.Trm	II cpm 53	t-tr 1
IIdvm7	-	-	-	II dvm 42	t-s 1
IIdvm8	-	-	-	II ism 44	t-s 8, t-s 7 ?
IIdvm9	-	-	AN.Pm	-	t-p 3
IItpm1	-	-	BA.Pm	II tpm 46a	t-p 4, t-p 20
IItpm2	x	-	-	II tpm 47	t-p 7, t-p 8, t-p 9
IItpm3	-	-	SrA.Pm, Ax.Pml	II tpm 46b ?	t-p 10, t-p 11, t-p 18
IItpm4	x	28	-	-	t-p 12
IItpm5	-	-	-	-	t-p 15
IItpm6	x	31	-	II tpm 49	t-p 13
IItpm7	x	33	-	II tpm 48	-
IItpm8	x	32	Ax.PmS	-	t-p 14
IItpm9	x	29/30	-	-	t-p 16
IItpm10	x	34	-	II ppm 56	t-p 19
IItpm11	-	-	-	-	t-p 17
IItpm12	-	-	-	-	p 1
IItpm13	-	-	-	?	?
IIppm1	-	-	-	II im 65a	p 2
IIppm2	-	-	-	II pm 54a, b	p 3
IIspm1	-	-	-	II ppm 55	p-s 1
IIspm2	-	-	-	II zm 61a	p-s 2

Tab. S1: Homologisation of thoracic muscle nomenclatures used by several authors

- absent / ? uncertain

IIspm3	-	-	-	-	p-s 6
IIspm4	-	-	-	-	p-s 7
IIspm5	-	-	-	-	p-s 3
IIspm6	-	-	-	-	p-s 9
IIspm7	-	-	-	-	p-s 5
IIspm8	-	-	-	-	p-ti(cx) 1
IIpcm1	x	21	-	-	p-ti(cx) 2, p-ti(cx) 3
IIpcm2	x	22	-	II cpm 51	p-cx 4, p-cx 6
IIpcm3	-	-	P.Cm	-	p-cx 5
IIpcm4	x	36	BA.Trm	II cpm 52	p-tr 2
IIpcm5	-	-	P.Trm	II cpm 50	p-tr 1
IIpcm6	x	39	Fm	-	s14, s16
IIvlm1	-	-	-	-	s 15
IIvlm2	-	-	iFm	-	s 13
IIvlm3	-	-	-	II vlm 38	s 11
IIvlm4	-	-	-	-	s 12
IIvlm5	-	-	-	II vlm 39	p-s 13
IIvlm6	-	-	-	-	p-s 10
IIvlm7	x	41	F.CmA	-	s-cx 5
IIscm1	-	-	-	II bm 57	s-cx 3
IIscm2	-	-	-	II bm 60	s-cx 6
IIscm3	x	38	F.CmP	II bm 59	s-cx 2
IIscm4	-	-	-	II zm 61b	s-cx 4
IIscm5	-	-	-	-	s-tr1
IIscm6	x	40	-	II bm 58	s-cx 1
IIscm7	-	-	-	-	s-cx 7
Metathorax					
IIIdlm1	x	45	MT.m	III dlm 35	t 14
IIIdlm2	x	45'	S.LPNm	III dlm 36, III dlm 37	t 12, t13
IIIdlm3	-	-	-	III dvm 40	?
IIIdvm1	x	46'	S.Esm	III dvm 41	t-p 5, t-p 6
IIIdvm2	-	-	S.CmA	-	t-ti 1, t-ti 2

Tab. S1: Homologisation of thoracic muscle nomenclatures used by several authors

- absent / ? uncertain

IIIdvm3	x	46	S.CmA	III dvm 43	t-ti 3
IIIdvm4	x	48	-	III dvm 43	t-cx 5
IIIdvm5	x	49	PSL.Cm, S.CmP	III cpm 53	t-cx 6, t-cx 7
IIIdvm6	-	-	SA.Cm, SA.Fm	III dvm 42	t-cx 8
IIIdvm7	-	-	-	III ism 44	t-tr 1
IIIdvm8	x	67	-	III tpm 46a	t-s 1
IIItpm1	-	-	AN.Pm	III tpm 47	t-p 3
IIItpm2	x	-	BA.Pm	III tpm 46b	t-p 4, t-p 20
IIItpm3	-	-	-	-	t-p 7, t-p 8
IIItpm4	x	50	SrA.Pm, Ax.Pml	-	t-p 10, t-p 11, t-p 18
IIItpm5	-	-	-	III tpm 49	t-p 12
IIItpm6	x	53	-	III tpm 48	t-p 15
IIItpm7	x	55	-	-	t-p 13
IIItpm8	x	54	-	-	-
IIItpm9	x	51/52	Ax.PmS	III ppm 56	t-p 14
IIItpm10	x	56	-	-	t-p 16
IIItpm11	-	-	-	-	t-p 19
IIItpm12	-	-	-	-	t-p 17
IIItpm13	-	-	-	?	?
IIIppm1	-	-	-	III im 65a	p 1
IIIppm2	-	-	-	III ppm 54a, b	p 2
IIIspm1	-	-	-	III ppm 55	p 3
IIIspm2	-	-	-	III zm 61	p-s 1
IIIspm3	-	-	-	-	p-s 7
IIIspm4	-	-	-	-	p-s 9
IIIspm5	-	-	-		p-s 5
IIIspm6	-	-	-	?	?
IIIpcm1	x	43	-	-	p-ti(cx) 1
IIIpcm2	x	44	-	III cpm 51	p-ti(cx) 2, p-ti(cx) 3
IIIpcm3	-	-	-	-	p-cx 4, p-cx 6
IIIpcm4	x	58	P.Cm	III cpm 52	p-cx 5
IIIpcm5	-	-	BA.Trm	III cpm 50	p-tr 2
IIIpcm6	x	62	P.Trm	-	p-tr 1

Tab. S1: Homologisation of thoracic muscle nomenclatures used by several authors

- absent / ? uncertain

IIIpcm7	-	-	-	-	p-cx 8
IIIvlm1	-	-	Fm	-	s 14, s16
IIIvlm2	x	68	-	III vlm 64	s 20
IIIvlm3	x	66	-	-	s 12
IIIscm1	-	-	F.CmA	III bm 57	s-cx 5
IIIscm2	-	-	-	III bm 60	s-cx 3
IIIscm3	x	61	-	III bm 59	s-cx 6
IIIscm4	-	-	F.CmP	-	s-cx 2
IIIscm5	-	-	-	-	s-cx 4
IIIscm6	x	63	-	III cpm 58	s-tr 1

4. Analysis System for Taxonomic Identification of Insecta Species Applicable to Strongly Degraded DNA Using the Nuclear 28S-rRNA Gene

4.1. Contribution to this Manuscript

Conceived and designed the experiments: PvG SB GT TH SH.
Performed the experiments: PvG SB JM.
Analyzed the data: PvG SB TH SH.
Contributed reagents/materials/analysis tools: SB GT TH SH.
Wrote the paper: PvG SB TH SH.

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Analysis system for taxonomic identification of Insecta species applicable to strongly degraded DNA using the nuclear 28S-rRNA gene

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ABSTRACT

In numerous contexts, e.g. biodiversity research or forensic entomology, fast and reliable species-level identification of insect specimens or even remains of insects is of high importance. Classical morphological methods of determination are often time-consuming and require expert knowledge, whereas identification via CO1-barcoding does not work reliably for certain taxa or for unfavourably preserved specimens.
Here, the introduced analysis system allows species identification of insect specimens or parts and can be applied on strongly degraded DNA. Instead of using mitochondrial sequences, the system focuses on the nuclear 28S-rRNA gene. The primers are designed to discriminate against human DNA and were tested on selected species covering all insect groups historically ranked as orders. Additionally, the samples feature a selection of various, mostly DNA-degenerating, preservation methods, including the most common fixation methods used in morphological investigations and storage methods used in collections.
In some cases, a single short sequence of the 28S-rRNA analysis system presented here already enabled reliable species-level identification of the specimen investigated. However, many of the obtained sequences are not found in the NCBI database and BLAST analysis revealed only close related species. The presented system could be a useful tool for species identification and a valuable addition to CO1-barcoding.

KEY WORDS

Addition to CO1, barcoding, degraded DNA, Insects, nuclear 28S rRNA gene, taxonomic identification

INTRODUCTION

The zoological collections of universities and museums harbour enormous numbers of preserved insect specimens from hundreds of years of expeditions. These collections enable an invaluable insight into biodiversity and pinpoint important areas of conservation.

In the fields of evolutionary and biodiversity research, molecular approaches have gained in importance in the last decade (e.g. Hebert und Gregory, 2005; Smith et al., 2006; Ward et al., 2005; Witt et al., 2006). Until now, only a few investigations (e.g. Chapco & Litzenberger, 2004; Goldstein & Desalle, 2003; Willerslev et al., 2007) have used the enormous amount of information available in the ancient DNA preserved in entomological collections. For example, museum specimens of the Adonis blue butterfly (*Polyommatus bellargus* Rottemburg, 1775) were analysed and revealed an extreme genetic drift in the populations (Harper et al., 2003). Further, Hartley et al. (2006) demonstrated that blowflies were preadapted for the rapid evolution of insecticide resistance. Recent advances in technical approaches for molecular biology

even provide a non-destructive method for extracting DNA from historical specimens (Gilbert et al., 2007; Thomson et al., 2009). Despite those promising results, studies using ancient DNA (aDNA) in entomological biodiversity research remain rare. Here, a PCR-based, modular analysis system for species-level identification of insect species is introduced that is specifically designed for application on degraded DNA. As Gilbert et al. (2007) reduced the damage for the initial DNA extraction from preserved insects; the analysis system proposed here reduces the following necessary analysis steps for taxonomic identification to a minimum. Thus, it meets the requirements of sustainable handling of preserved specimens from zoological collections. Additionally, the system is applicable in all contexts that have to deal with greatly degraded DNA, like forensic contexts (e.g. Ferri et al., 2009), processed material (Meusnier et al., 2008), or inadequately collected specimens (e.g. Büsse et al., 2012). In order to achieve this, three major aspects have to be considered in the field of aDNA research: DNA degradation; only small amounts of DNA in a fit state for analysis; and possible contamination with exogenous DNA.

A limiting condition is the maximum length of DNA obtainable from preserved specimens. In studies that investigated the evolution and relationships of species (e.g. Carle et al., 2008; Ishiwata et al., 2011; Kjer et al., 2006; Saux et al., 2003) or dealt with the possibility of taxonomic identification of species based on barcoding different genes (e.g. Hajibabaei et al., 2006; Hebert et al., 2003a), for example, amplified fragments were usually longer than 500bp. Shorter fragments allow a broader application of DNA barcoding while still enabling reliable identification in up to 90% of the samples (Hajibabaei et al., 2006; Meusnier et al., 2008). Further, in specimens stored for a long time, one of the most important aspects is the likelihood of contamination with exogenous DNA, such as human DNA from earlier investigators (e.g. Hebsgaard et al., 2005). Thus, the primers used here are designed to discriminate against human DNA.

The mitochondrial cytochrome oxidase subunit I (COI) is usually used for taxonomic identification (e.g. Gomez et al., 2007; Hebert et al., 2003b), although alternatives like NADH dehydrogenase subunit I (Rach et al., 2008) have been discussed. However, identification by means of the sequence of COI does not work for all groups of insects (e.g. Herrera et al., 2010; Whitworth et al., 2007) and might face unexpected challenges (Smith et al., 2012). Further, most of the COI sequences of insects are often identified only to order (Kwong et al., 2012) and nuclear genes might reveal higher resolving power than mitochondrial genes (Feau et al, 2011).

In the analysis system proposed here, short polymorphic regions of the nuclear 28S-rRNA gene are used. These genes are usually analysed in evolutionary and phylogenetic contexts and are already available for many insect species. In addition, they reveal

both highly polymorphic as well as conservative regions, which are necessary for designing primers (see below). Other genes like 5,8S or 12S-rRNA show low variability and thus do not allow a clear distinction of species. The more variable 18S-rRNA gene allows identification on the genus level, while the most variable 28S-rRNA gene enables reliable discrimination on the species level. The length of the amplified regions can be adapted to the degree of DNA degradation and varies between 110bp and a maximum of 290bp.

If DNA is available only in trace amounts, successful investigation requires very efficient analysis parameters. Therefore, primers are designed to match the criteria for ancient DNA analysis (cf. e.g. Hummel, 2003), which are optimized with respect to sensitivity and specificity.

METHODS AND MATERIAL

Primer Design

The investigated sequences should show a high diversity among the species, thus enabling reliable discrimination. Furthermore, to achieve an amplification of as many insect species as possible, the investigated polymorphic regions should be flanked by highly conservative sequences at which the primers hybridize. To gain information about variable regions, sequences of insect species were aligned using the program MegAlign (www.DNAStar.com) and the Clustal V algorithm. A total of four regions on the 28S-rRNA gene were found (cf. Figure 1A) that show these characteristics. Primers were designed for all sites using the program PrimerSelect (www.DNAStar.com) and following the criteria for ancient DNA analysis (e.g. Hummel, 2003). For example, for a higher specificity, the 3' ending of the primer should have a lower binding energy than the 5' ending to allow the Taq-polymerase-driven elongation process only if the whole primer hybridized completely. For optimal sensitivity, primer dimers and hairpins must be avoided. Regarding DNA degradation, the length of the amplification products should be as short as possible with a maximum of 300bp. For two sites (28S3 and 28S4), primers were designed that can be combined depending on the degree of DNA degradation (cf. Figure 1B). The specificity of the primers for discrimination against human DNA was first checked by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and PrimerBLAST analysis (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). Primer sequences and optimal annealing temperatures are shown in Table 1.

Primer Testing

As revealed by alignment analyses, the site 28S4 seemed to be the most polymorphic site and often species specific (cf. Figure 1C), despite the fact that this site is one of the shortest in this study and thus best suited for aDNA analyses. To investigate the discrimination power for 28S4, the usability for aDNA application and the limits of

the proposed primers, the longest and shortest primer combination of 28S4 (cf. Table 1) was tested on 65 insect species and 13 other arthropod species; in total, 82 specimens varying in age and preservation status were investigated (Table S1). The sample selection contains exemplar species from 48 Arthropod taxa including all insect taxa historically ranked as orders, to show the functionality of the universal insect primers throughout Insecta. Species from different major subgroups of certain taxa like the odonatan Anisoptera (*Libellula depressa*), Zygoptera (*Coenagrion spec.*) and *Epiophlebia* (*E. superstes*) were included to show the differentiability on this degree of relationship. Further, a couple of more closely related species, for example within Cupedidae (Coleoptera: Archostemata) and within Phasmatodea, as well as conspecific specimens from the same population were included. This study also contains phylogenetic key species like Cryptocercus (Blattodea) and Mastotermes (Isoptera), as well as taxa whose phylogenetic position was recently under discussion, like Embioptera and Phasmatodea (e.g. Inward et al., 2007; Ishiwata et al., 2011; Letsch et al., 2012). Specimens from other arthropod groups include Remipedia (Crustacea), which is a sister group candidate for the Insecta (e.g. Regier et al., 2010).

Additionally, the samples comprise a set of various preservation methods, which often degenerate the DNA. For example, morphological investigations often require tissue fixation using fixatives that contain formaldehyde, like Bouin, FAE or Carnoys (Romeis, 1989). Storage in museums also requires special treatments like drying or storage in liquid mixtures often also containing formaldehyde (e.g. Romeis, 1989). The age of the specimens ranged between recently collected to approximately 200 years old. The investigated museum samples were all donated by the Zoological Collection of the University of Goettingen.

Before DNA extraction took place, the guts of the specimens were removed in order to prevent contamination from prey; only the thorax and the leg muscles were used. The analyses were done at the aDNA laboratories of the Institute of Historical Anthropology; and commonly applied precautions to avoid contamination were taken (e.g. separation of pre- and post-PCR laboratories and the use of disposable protective clothing, security glasses and disposable gloves, cf. Hummel, 2003).

DNA extraction began with a cell lysis with a first step of mechanical tissue shredding. At least 200µl and up to 600µl ATL buffer (Qiagen, Germany) was added depending on sample weight. 30µl of Proteinase K (20mg/ml) was added to the solution and incubated at 56°C for 18 hours under constant agitating. 200µl of the supernatant was used for automated DNA extraction with the Biorobot® EZ1 (Qiagen, Germany) following the protocol of the Forensic protocol- procedure. The elution volume was 100µl; the DNA extract was stored at -20°C.

In PCR reactions, the volume in each setting was 25µl, containing 12.5µl of master mix (Qiagen 2x MasterMix plus), 0.4µM of each primer and 0.5 – 5µl of DNA extract, filled up with RNAse free water (Qiagen). PCR was carried out under the following conditions: initialization 95° C for 5 min; 40 cycles at 94°C for 1 min, annealing temperature (see below) for 1 min, 72° C for 2 min; final elongation at 72°C for 7min; and soak at 10°C for 10min. The annealing temperature was set between 55°C and 68°C, depending on the species and the occurrence of secondary products. Afterwards, the PCR success and product quantity were checked by agarose gel electrophoresis. Negative controls as well as human control DNA were also included.

Further purification and sequencing were carried out with commercial kits (MiniElute® PCR Purification Kit, Qiagen, ABI Prism BigDye V 3.1 Terminator Cycle Sequencing Kit and NucleoSeq Kit, Macherey-Nagel) as specified by the manufacturers. Both the forward and reverse amplification primers were also used for the sequencing reaction. The sequencing conditions were: initial at 96°C for 10min, 25 cycles at 96°C for 10s, 50°C for 5s and 60°C for 4min. For sequence analysis an ABI 310 genetic analyser with POP6 polymer was used. Sequence reads were checked for quality and assembled using SEQMAN (Lasergene; www.DNASTAR.com). Sequences were checked for specificity through BLAST (http://blast.ncbi.nlm.nih.gov/).

Phylogenetic Analysis

To evaluate the information on phylogenetic relationships provided by the sequences investigated here, we compiled several different datasets for phylogenetic analysis. The sequences were aligned using MEGA version 5 (Tamura et al., 2011) and automatic alignment with the clustalW algorithm and standard parameters. The resulting alignments were subsequently checked and corrected manually.

The most appropriate models of DNA substitution for Bayesian and maximum likelihood tree searches were selected with MrModeltest 2.3 (Nylander et al., 2004) and PAUP*4.0b10 (Swofford et al., 2001) separately for each sequence alignment. In addition to a dataset with representatives from all major taxa (57 OTUs, Archaeognatha as outgroup), matrices of different sizes were analysed representing Dictyoptera, Coleoptera, Mecopterida, Polyneoptera, Phasmatodea and basal splittings in Pterygota. Phylogenetic relationships were calculated with the maximum likelihood (ML) algorithm in PAUP*.

RESULTS

Despite the variable conservation status and age, all samples of the tested Insecta taxa showed specific amplification products for the tested site although in some cases adjustments concerning annealing temperature were necessary. In a few cases only one of the two primer combinations (cf. Figure 1) revealed products that could

be sequenced, suggesting that primer adjustments might be necessary for optimized analysis. Primers for the short amplification product revealed results in nearly all species investigated. For this primer pair, amplification products vary in length from ~110 to 240bp between species. However, the proposed primers seem to be applicable to a broad range of Insecta species. Amplification of the additional Arthropod species was successful in ten of 13 cases.

Regarding the NCBI database, in some cases even the obtained single sequences enabled taxonomic identification of species (cf. Table S1). For example, all tested Odonata species were clearly distinguishable. However, in most cases the BLAST analysis revealed only similar but not exactly the same sequence. This allowed only identifying related species. Further, well investigated genera like Oligotoma and Metoligotoma (Insecta: Embioptera) show that some species revealed the same sequence at the 28S4 site and thus a combination with other sites, e.g. 28S3, is necessary to enable an unambiguous identification.

Amplification of the 28S4 locus of the additional Arthropod species (like crustaceans, spiders and millipedes e.g. Table S1) with the proposed primers was more difficult and often needed a reduced annealing temperature. Further, in some species the locus revealed the same sequence although these were not closely related (cf. Table S1). This might indicate that the applicability of the analysis system is limited to Insecta species and postulates an adaptation of the primers for other Arthropod species. Our tests of phylogenetic analyses based on the 28S4 sequence only resulted in relationship hypotheses that are not compatible with any published phylogenies. This includes all combinations of taxa that were analysed. Arrangements of taxa on the resulting trees appeared to be random and depended heavily on the outgroup used. Such a result was to be expected, since the 28S4 sequence is comparatively short and highly variable. The extent of this variability becomes clear when closely related species are compared. E.g. in the species of Archostemata (Coleoptera) investigated here, Ascioplaga mimeta Nebpiss, 1984 has an insertion of 201 base pairs that was found in no other species. There even seem to be no patterns that are unique to Coleoptera. On the other hand, Amphiesmenoptera (Lepidoptera + Trichoptera) were monophyletic in all our analyses. While heavily distorting reconstructions of phylogenetic relationships, such a high level of variability is very helpful for the unequivocal identification of species.

DISCUSSION

In this study, we introduced a modular, PCR-based method for species identification in Insecta, which is especially helpful in investigating strongly degraded ancient DNA of preserved specimens. The testing of the most polymorphic site 28S4 on a broad range of species and the fact that the sequences of newly investigated species did

not reveal perfect matches in NCBI shows that this system has the potential to be a powerful tool in taxonomic identification in various kinds of research fields. Due to the diversity of the insect taxa and the limited databases on which the introduced system is based, the taxonomic identification based on only one short sequence has its limitations. In some species tested the additional sequence of 28S3 was necessary for unambiguous taxonomic identification. Thus, the combination of several short sequences of the nuclear 28S gene and the ever-expending databases promise that the system can be a useful alternative to mitochondrial CO1 barcoding, especially in those cases in which this marker does not allow a taxonomic identification or in which DNA degradation took place (cf. Fig. 2).

However, the 28S4 sequence alone is not useful for phylogenetic analyses, as our test clearly demonstrated. This section of the 28S gene is far too variable to contain enough stable information for the reliable reconstruction of phylogenetic relationships on any taxonomic level. Nevertheless, this variability facilitates the identification of species. The sites 28S1 and 28S2 were not practically tested in this study, but might be useful in cases in which the other two sites fail.

Some precautions have to be considered when using universal primers as introduced here: I. Although the primer design is based on the large NCBI database, due to the larger diversity of Insecta mutations at the primer hybridization sites might be occuring. Such mutations may cause PCR failures. Therefore, for two regions (28S3 and 28S4), alternative primers that can be used in different combination are proposed. Based on the 28S4 tests proposed primers seem to be applicable on a broader range of species. However, to ensure efficient analyses primer adaptions might be necessary. Further, adapted primers ensure sensitivity and efficiency to samples with low DNA content. II. The proposed annealing temperature can also be modified. A lower annealing temperature may enable amplification even if there is a mismatch in the primer hybridization site. However, in principle, this type of modification of PCR parameters reduces the specificity of the primers. Still, the high discrimination against human DNA of the primers presented here generally allows temperature reduction without risking amplification of contaminating human DNA. III. The use of universal primers makes it necessary to avoid contamination with other insect DNA, which would lead to mixed sequences (e.g. Bourlat et al, 2003; Gee, 2003).

Nonetheless, this method provides access for all kinds of fields dealing with degraded DNA and the biodiversity of Insecta and is a useful addition to CO1 barcoding.

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Figures

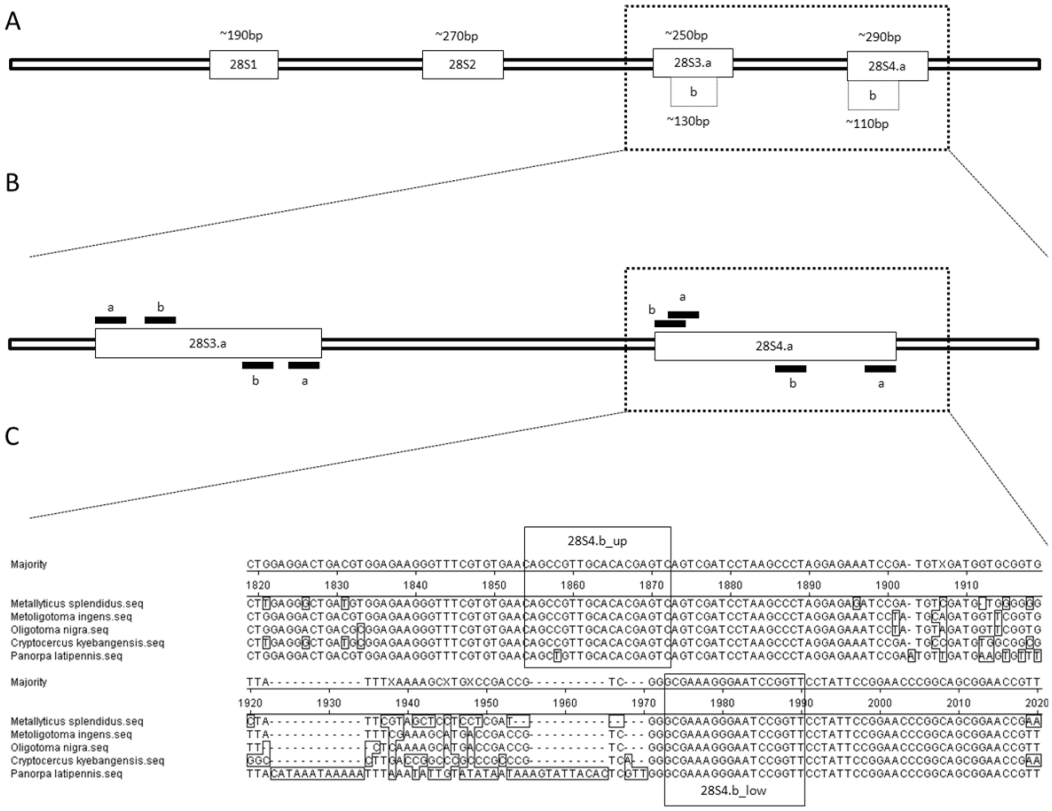

Figure 1: A. Approximate location and length of the four chosen sites. B. Primer locations on two of the sites. The primers can be used alternatively and allow an adaption to the degree of DNA degradation. C. Alignment example of five randomly chosen species. Encircled are the positions of the primers. The sequence in between was revealed to be highly polymorphic and thus enables species identification. Sequences used for alignment: FJ806598.1 (M. splendidus), EU157057.1 (M. ingens), AY125274.1 (O. nigra), FJ806522.1 (C. kyebangensis), AF423964.1 (P. latipennis).

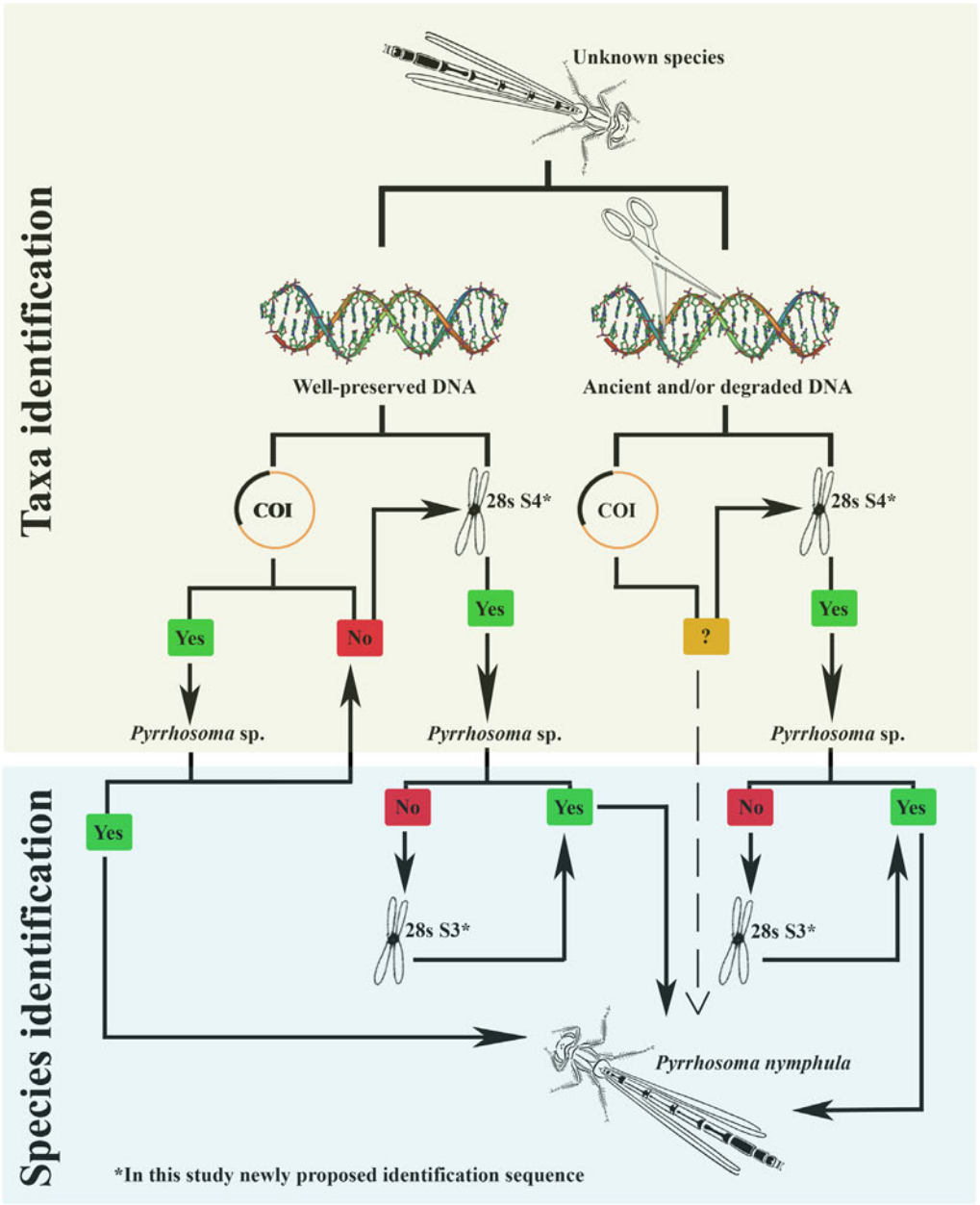

Figure 2: Schema of proposed proceeding when identifying insect species. As addition on the mitochondrial CO1 gene the short sequences of 28S can be used.

TABLE

Table 1: Primer sequences and proposed annealing temperatures. (* cf. Büsse et al. 2012)

Primer	Sequence (5'-3')	Length (bp)	Annealing temperature
28S1_up*	TCGGACACGCTCCGCTAAAC	~190	64°C
28S1_low*	GCCAGGCATAGTTCACCATCTTTC		
28S2_up*	CCGGTAAAGCGAATGATTAGAG	~270	60°C
28S2_low*	CCACCGTCCTGCTGTCTTAA		
28S3.a_up*	GGAATCCGCTAAGGAGTGTGTAA	~250	58°C
28S3.a_low*	AGGGCCTCGCTGGAGTATTT		
28S3.a_up*	GGAATCCGCTAAGGAGTGTGTAA	~190	58°C
28S3.b_low	GCTCGAGCCCAGACCCTT		
28S3.b_up	GCTGAAGCGTCGTGCCTATAC	~190	58°C
28S3.a_low*	AGGGCCTCGCTGGAGTATTT		
28S3.b_up	GCTGAAGCGTCGTGCCTATAC	~130	58°C
28S3.b_low	GCTCGAGCCCAGACCCTT		
28S4.a_up*	CCGTTGCACACGAGTCAGTC	~290	58°C
28S4.a_low*	TCGCGTTCCAAACCCTATCT		
28S4.a_up*	CCGTTGCACACGAGTCAGTC	~110	58°C
28S4.b_low	AACCGGATTCCCTTTCGC		
28S4.b_up	ACAGCCGTTGCACACGAGTC	~290	58°C
28S4.a_low*	TCGCGTTCCAAACCCTATCT		
28S4.b_up	ACAGCCGTTGCACACGAGTC	~115	58°C
28S4.b_low	AACCGGATTCCCTTTCGC		

5. The Thorax Morphology of Zygoptera (Insecta: Odonata) - The Skeletal System.

5.1. Contribution to this Manuscript

Conceived and designed the experiments: CG SB TH.
Performed the experiments: CG TH.
Analyzed the data: SB CG TH.
Contributed reagents/materials/analysis tools: CG SB TH.
Wrote the paper: CG SB TH.

5.2. Manuscript

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The thorax morphology of Zygoptera (Insecta: Odonata) - the skeletal system.

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Abstract

The flight skills of dragonflies and damselflies (Odonata) are astonishing and quite unique among the winged insects (Pterygota). They are aerial predators that hunt and catch their prey in flight, only. Not only the flight skills but also the morphology of the flight apparatus of Odonata is significantly different from what is found in the remaining Pterygota. Especially because the flight apparatus seems to be so unique, it is essential to know how the elements of the odonatan flight apparatus relate to those of the other Pterygota in order to understand the phylogenetic relationships of winged insects and the origin and evolution of insect flight in general. Here we present a comprehensive, comparative investigation of the thoracic sclerites of damselflies (Zygoptera) focusing on the elements of the wing base and its related muscles, with a discussion of the homologization of these sclerites between Odonata and Neoptera.

Keywords: Odonata, dragonflies (Anisoptera), damselflies (Zygoptera), flight apparatus, wing base sclerites

1 Introduction

The acquisition of wings is one of the key factors of the evolutionary success of insects. This is documented by the fact that Pterygota comprise more than 98% of all known insect species. (Grimaldi & Engel 2005, Hörnschemeyer & Willkommen 2007).

The Odonata comprising the Zygoptera (damselflies) and the Anisoptera (Dragonflies) plus the enigmatic *Epiophlebia*, are special in comparison to the remaining Pterygota (Ephemeroptera and Neoptera), because they are extremely agile aerial predators with a very special version of the insect flight apparatus. In contrast

to the other Pterygota, the flight musculature of Odonata has no, or at least highly reduced, dorsal longitudinal muscles (Willkommen 2008), which are the dominant muscles in the pterothorax of Ephemeroptera and Neoptera, because these are responsible for the power stroke of the wings in their indirect flight motor system (e.g. Snodgrass 1935). In Odonata the muscles and sclerites are rearranged to form a direct flight motor system where mainly dorso-ventral muscles are attached to the wings in such a way that each wing can be moved directly and independently from the others; a system that gives for a unique manoeuvrability to these aerial hunters. Striking features of skeletal elements are the comparatively short and high pterothoracic segments and the wing base that comprises two large sclerites that do not allow for a backward flexing of the wings, as it is possible in the Neoptera (Tannert 1958, Pfau 1986, Willkommen & Hörnschemeyer 2007, Ninomiya & Yoshizawa 2009).

Structures of the flight apparatus of Odonata have been investigated in some detail already (e.g. Tannert 1958, Pfau 1986, Willkommen & Hörnschemeyer 2007, Willkommen 2008, Ninomiya & Yoshizawa 2009, Büsse et al. 2013), including some suggestions for the homologization of skeletal elements of the flight apparatus in Odonata and Neoptera. However, the earlier investigations are mainly based on species of Anisoptera. To improve and broaden the knowledge on Odonata thoracic morphology we focus on species of Zygoptera. In contrast to the previously dominating opinion (e.g. Matsuda 1970) that the wing articulation of Odonata and Ephemeroptera represents a plesiomorphic state, the younger investigations arrive at the conclusion that the flight apparatus of these taxa is highly modified, i.e. derived. In contrast to all remaining Pterygota, the musculature of Odonata actuates the wings directly (Snodgrass 1935, Büsse et al. 2013). This direct actuation is also apparent in the structures of the wing articulation of Odonata (Tannert 1958, Pfau 1986). The, at first sight, substantial differences in the sclerites and muscles of the flight apparatus between Odonata and the other pterygotes makes the homologization of these structures difficult. However, Büsse et al. (2013) offered a first consequent hypothesis for the homologization of the thoracic musculature of Neoptera and Odonata and a convincing homologization of the thoracic and the wing base sclerites between Odonata and Neoptera would open up an extensive new set of characters for phylogenetic analyses an thus facilitate a better understanding of insect evolution.

2 Material and Methods

Adult specimens of the following species of Zygoptera have been investigated:

Coenagrionidae

- *Pyrrhosoma nymphula* (Sulzer, 1776): Billingshaeuser Schlucht, Goettingen, Germany
- *Coneagrion puella* (Linnaeus, 1758): Billingshaeuser Schlucht, Goettingen, Germany
- *Enallagma cyathigerum* (Charpentier, 1940): Billingshaeuser Schlucht, Goettingen, Germany
- *Ischnura elegans* (Vander Linden, 1820): Billingshaeuser Schlucht, Goettingen, Germany

Calopterygidae

- *Calopteryx splendens* (Harris, 1782): Billingshaeuser Schlucht, Goettingen, Germany and Villemur sur Tarn, France

Platycnemididae

- *Platycnemis latipes* (Rambur, 1842): Barsac, France

- *Platycnemis pennipes* (Pallas, 1771): Barsac, Frankreich and Villemur sur Tarn, France

Lestidae

- *Lestes viridis* (Vander Linden, 1825): Barsac, France

All regulations concerning the protection of free-living species were followed. Permits for collecting species of Odonata were obtained from the appropriate authorities.

The specimens were collected at the above mentioned sites and transported in 80% EtOH. Subsequently, they were fixed in Dubosq-Brasil fixative (Romeis 1989) and stored in 80% EtOH.

To make the drawings a stereomicroscope (Zeiss Stemi SV11) with camera lucida was used. The specimen was bisected longitudinally. The photographs were made with a Keyence digital microscope VHX600 at the department of mineralogy at the Georg-August University, Goettingen, Germany.

Synchrotron radiation micro computed tomography (SRμCT) data where acquired at the Swiss Light Source (SLS) in Villingen (Switzerland), using the beamline Tomcat, (Proposal no. 20080794, Mai 2009, TH) and (Proposal no. 20100088, Nov. 2010, TH) well as at the Deutsches Elektronen Synchrotron (DESY) in Hamburg, Germany, (Proposal no. I-20090102, SB). Prior to CT-scanning, samples were critical point dried (CPD) (BalzerCPD030). Three-dimensional reconstructions (processing and visualization) of the data were prepared with Amira® 5.2. (Visage Imaging, Richmond, Australia). All output images were subsequently processed in Photoshop CS3 (Adobe System Inc., San José, USA).

A list of the used abbreviations is additionally available as Supplement (Suppl. 8).

3 Results

The investigation is based on *Pyrrhosoma nymphula*, which is used as a reference with which the other species are compared. The thoracic musculature has been described in detail in Büsse et al. (2013).

3.1. Morphology of the pterothoracic tergum

The arrangement of the tergal sclerites is similar in all species studied. The acrotergite is missing in all species. The main differences between species can be found in the extend of the sclerotisazion, in the coloration and to some degree in the shape of scerites, which is most conspicuous in the prescutum. The degree of sclerotization seems to depend on the body size of the species, since smaller species appear to be less sclerotized (Suppl. 6, 7).

The tergal sclerites are cranially and laterally connected to the pleurum or to the wing via membranous areas. The postnotum 3 has a membranous connection to the 1st tergite of the abdomen. The two scutal plates are located bilateral of the scutum and belong to the prescutum (Suppl.5: β & τ; π & λ).

3.1.1. Tergum of the mesothorax

The origin of the 3. tergal apophysis is located anteriorly in the central part of the scutum (Fig. 1 A & C) (Suppl.1, 3, 4, 5, 6, 7). This apophysis protrudes thorn-shaped into the thorax and serves as muscle point of attachment for muscle IIdlm1. Bilateral of the origin of the 3rd tergal apophysis (Fig. 1 τ) are the attachment

points of muscles IItpm4. The median part of the scutum is convex and prominent. In Coneagrionidae this part is ovoid (Fig. 1 A & C) (Suppl.1, 3, 4). In *C. splendens* it is narrowest (Suppl. 5). In Platycnemididae, it is less convex, wider and has a shallower relief than in all other species studied. In *L. viridis* the convex part is trapezoid.

At both sides of this convex part of the scutum apodems are present that serve as muscle attachment points of muscles IIdvm3. Caudal the scutum ends with a strongly sclerotised, bulging terminal ridge (Suppl. 4) that is somewhat darker in colour. In *I. elegans* it is conspicuously thick and dark. The lateral ends of the scutum articulate with the fused radius – media veins forming the proximal corners of the axillary plate 2.

Like the scutum, the scutellum has a central, distinctly convex region. This convexity is circular, higher and lighter in colour than the one of the scutum (Suppl.1, 3, 4, 5, 6, 7: è). Caudal the scutellum ends merges into the flexible axillary cord, which is connected to the anal vein. Bilateral the scutelum is connected to the axillar plates.

The postnotum is wider than the scutellum and establishes a flexible connection between meso- and metanotum. There are no muscles connected to the postnotum. It is partially folded under the scutellum, especially in *I. elegans*. Medially it is slightly convex and lateral it is connected to the pleura. In *L. viridis* the postnotum has a bilaterally extending membranous area that reaches to the pleura. In *P. pennipes* and *P. latipes* the postnota seem to be generally less sclerotized and more transparent than in the other species investigated.

3.1.2. Tergum of the metathorax

In the metathorax the scutal plates, the scutum, the scutellum and the postnotum all are slightly longer than in the mesothorax resulting in a generally slightly longer metatergum. In contrast to the mesotergum the scutum of the metatergum has a narrow, weaker sclerotized band that runs across the width of the tergum. Medially, the metascutum has two convex areas, instead of one in the mesotergum. Muscle IIIdvm3 has a wide attachment point at the scutum (Fig. 1 B & D) (Suppl. 1, 3, 4, 5, 6, 7). The two partners of muscle IIIdlm2 have their points of attachment on both sides of the origin of the 4th tergal apophysis. The membranous postnotum connects the scutum to the 1st tergite of the abdomen. In contrast to the mesothorax it is not folded under the scutellum. In *P. pennipes* and *P. latipes* the postnotum is so weakly sclerotized and transparent that the two partners of muscle IIIdlm2 that are passing beneath are distinctly visible through the cuticle.

3.1.3 Prescuta

In the Zygoptera investigated the prescutum is neither distinct visible nor it is delimited from the scutum by a transversal suture. In both segments of the pterothorax the prescutum is located directly in front of the origin of the tergal apophyses and streches laterally to the scutal plates. The prescutum is smaller in the mesothorax than in the metathorax. In *P. nymphula*, *C. puella*, *E cyathigerum* (e.g. Fig. 2C) and *L. viridis* it is almost invisible.

The scutal plates are longer in the metathorax than in the mesothorax. Hatch (1981) as well as Ninomiya & Yoshizawa (2009) identifies three separate regions in each scutal plate: the caudal, median and distal semi-

detached scutal plates. The median and distal semi-detached scutal plates are not separated from each other by sutures in any of the species investigated. The distal semi-detached scutal plate lies adjacent to the axillar plate and the median semi-detached plate adjoins the scutum. Here we follow the definition of Ninomiya & Yoshizawa (2009).

C. splendens and the Platycnemididae show these structures most distinctly (Fig. 2B, 2D) (Suppl. 5). In all Zygoptera the attachment point of muscle IIdvm1 is located on the scutal plate (Fig.1B) (Suppl.5) and at its posterior lateral margin is the attachment point of muscle IIdvm4.

This division of the scutal plate into three parts is not visible in *L. viridis* (Fig. 2E). However, also the scutal plate of this species has a small lateral bulge that is present in all species investigted on the median semi-detached plate. In *C. splendens* the shape of the scutal plate is more irregular and somewhat serrated. Its median semi-detached plate of the mesothorax has a circular depression (Fig. 2B), which is not present in the metathorax. In *Coenagrionidae* the caudal semi-detached scutal plate lies more laterally.

3.2 Morphology of the ptero-thoracic pleura

In Odonata the pleura delimit the mesotergum not only laterally, as in other insects, but also cranially where the mesepisterna merge to form the pleural keel. The typical odonatan dorsoventral expansion of the pleura is present in all species investigated.

The pleura of *C. splendens* are wider than in all other species investigated, therefore the pterothorax appears to be more massive and less elongated.

In some species of Anisoptera the pleural sutures show a ladder like structure (Russenberger & Russenberger 1960), which could not be observed in the Zygoptera. To the corresponding internal pleural ridges several muscles are attached (IItpm2, IItpm6, IIItpm2, IIItpm6). In *C. splendens* the pleural ridge is broader than in other species. Dorsally, the pleural ridge merges into the pleural processes. The segmental border between mesepimeron and metepisternum is present as consistent ventro-dorsal suture in all Zygoptera, whereas, in the Anisoptera this suture is visible only at the base of the pleura (Tannert 1958). Ventrally, the epimeron and the episternum are adjacent to the katepisterna 2 and 3. Several muscles are attached to the ridges between epimeron and katepisterum (IItpm7 (IIItpm7), IItpm8 (IIItpm8), IIpcm4 (IIIpcm4), IIpcm6 (IIIpcm6)).

4 Discussion

The muscle names given in braces refer to muscles of the metathorax that correspond to the mesothoracic muscles.

4.1. The prescutum and the scutal plate

Among the tergal sclerites, the prescutum is the element that shows the highest variability between species. In the Zygoptera the prescutum comprises a narrow central part in the anterior margin of the notum that is located directly above the tergal apophysis and extends laterally to the scutal plates, which are unique among Pterygota. Structurally the scutal plates also belong to the prescutum (Willkommen 2008). They are the attachment points of the muscles IIdvm1 (IIIdvm1), IIdvm4 (IIIdvm4) and IItpm4 (IIItpm4).

The association and origin of the odonatan mesoprescutum have been discussed by several authors: Asahina (1954), Garman (1916) and Grandi (1947) assumed a connection of the mesoprescutum with the tergal apophysis 3, which Tannert (1958) contradicted. He saw a close association with, and probably an origin of the mesoprescutum from, the protergum. Pfau (1986), however, identified the mesoprescutum as the sclerite from which the tergal apophyses 3 are formed.

The scutal plates can be diferenciated into three areas: the median, distal and caudal semi-detached plates Ninomiya & Yoshizawa 2009). In *L. viridis* these three elements are not clearly separated. In *C. splendens*, in Coenagrionidae and in Platycnemididae have a distinct caudal semi-detached plate. In Platycnemididae, however, it is shifted laterally (Fig. 1B). Generally, the scutal plates show a distinct variability between species.

4.2. Homology of the wing sclerites of Zygoptera and Neoptera

4.2.1. Humeral plate

The humeral plate is a small sclerite in the anterior border of the neopteran wing, which articulates with the base of the costa (Snodgrass 1935). The distal costal plate of Odonata is distally connected to the costal vein through two points of articulation. Proximally it borders the proximal costal plate. Thus, its homologization with the neopteran humaral plate appears to be highly probable (Tannert 1958, Henning 1969, Matsuda 1970, 1979, Pfau 1986, Brodsky 1994, Ninomiya & Yoshizawa 2009).

4.2.2. Basalare

As in all other Odonata the proximal costal plate in the specimens investigated is a wide plate in the dorsal wing base area that is located proximal of the distal costal plate, which, in turn, articulates with the costa. Two muscles are cranially attached to the proximal costal plate through tendons. Ninomiya & Yoshizawa (2009) hypothesized that this plate is homologous to the basalar sclerite of the Neoptera. The neopteran basalare has two muscles attached to it. Considering the points of origin and insertion of the two muscles that are attached to the proximal costal plate, it can be assumed that these are homologous with the neopteran basalar muscles IIpcm1 (IIIpcm1) and IIpcm2 (IIIpcm2). This, in turn, supports the assumption of the proximal costal plate being homologous with the neopteran basalare. However, the proximal costal plate is located in the dorsal wing base whereas the neopteran basalare is a pleural sclerite (Snodgrass 1935). At first look it might seem improbable that the basalare moved from the pleura to the dorsal side of the wing base (or in the opposite direction; depending on the sequence of evolution). But in Ephemeroptera the basalare sclerite is a brace like sclerite running from the anterior dorsal area of the wing base around the anterior border of the wing to the dorsal pleurum (Willkommen & Hörnschemeyer 2007, Willkommen 2008). This might well be an intermediate state between the neopteran and the odonatan situation. Therefore we interpret the proximal costal plate of Odonata as the homolog of the neopteran basalare.

4.2.3. Axillaries

In the Zygoptera investigated the axillary plate consists of a dorsal and a ventral sclerite (the epifulcrum),

which enclose the muscle IItpm9 (IIItpm9). Both sclerites fuse distally and merge into the radius, media, cubitus and anal veins. Several authors support the assumption that the axillary plate is homologous to the joint 1st, 2nd and 3rd axillaries of the Neoptera (e.g. Snodgrass 1935, Ninomiya & Yoshizawa 2009). There the 1st axillary is connected to the subcosta, the 2nd axillary to the radius and the 3rd axillary is connected to the anal veins. Snodgrass (1935), Chao (1954) and Tannert (1958) recognised a 1st axillary in the Odonata, even though, it has no direct connection with the subcosta. According to Matsuda (1970), this odonatan 1st axillary is not homologous to the 1Ax in the Neoptera. Furthermore, it is not possible to identify the 2nd and 3rd axillaries in the remaining area of the axillary plate. Similarly, an unequivocal identification of separate areas homologous to the 1st, 2nd and 3rd axillaries or the median plates was not possible in the Zygoptera investigated herein.

Cranially, the axillary plate is bordered by the fused radius-media vein and caudally by the anal vein. Its surface is slightly uneven. Proximally, it is stronger sclerotized and connected to the scutellum. In total, the arrangement of the sclerites and the association with the wing veins support the assumption that the axillary plate is homologous to the fused axillaries 1, 2 and 3 plus the median plate(s).

4.2.4. Subalare

The subalare sclerite of Neoptera is an unpaired pleural element of the wing base that usually is separated from the epimeron by a wide membrane.

In all species studied the pleural bar dorsally differentiates into the anterior and the posterior pleural process and the tergal lever (Fig. 2A). These three parts form a sclerite that is, depending on the species, more or less strongly connected with the epimeron through the tergal lever. The position of this sclerite resembles the position of the subalare in the Neoptera (Snodgrass 1935), thus, suggesting a homology of these sclerites. However, Snodgrass (1935) described a special subalarmuscle (1E') in Gryllidae, Trichoptera and Lepidoptera. Its point of origin is at the epimeron and the insertion at the subalare. In the Zygoptera studied the muscle IItpm9 (IIItpm9) attaches at the posterior pleural process via a cap tendon and the origin is at the caudal region of the axillary plate (Fig. 5A; Büsse et al. 2013). These points of origin and insertion suggest a homology with muscle 1E' of Snodgrass (1935). Considering the route of this muscle and the arrangement of the sclerites at which the muscle attaches, the posterior pleural processes together with the posterior tergal lever of Odonata are most likely homologous to the subalare of Neoptera, whereas the anterior pleural process of the Odonata is the homologous element of the neopteran pleural process.

Summarizing the available information, the homologization of most important structures of the flight apparatus of Odonata and Neoptera appears to be quite straightforward. Büsse et al. (2013) recently suggested a homologization scheme for the thoracic musculature of Odonata and Neoptera and with the information presented herein also important sclerites can be homologized with some confidence: the humeral plate of Neoptera is homologous to the distal costal plate of Odonata, as was already suggested by Ninomiya & Yoshizawa (2009). The proximal costal plate of the Odonata can be homologized with the neopteran basalare and there is some evidence that the neopteran subalare is equivalent to the posterior pleural process plus the posterior tergal lever of Odonata. For the axillary sclerites 1 to 3 slightly different homologizations

have been proposed by Willkommen & Hörnschemeyer (2007), Willkommen (2008) and Ninomiya & Yoshizawa (2009). The evidence from the present study gives support to the interpretation of Ninomiya & Yoshizawa (2009), assuming that the central axillary plate of Odonata is homologous to all three neopteran axillaries as well as the median plates. All this supports the assumption that the flight apparatus of Odonata is a highly derived version, specialized for aerial hunting, of the flight apparatus as it was present in the last common ancestor of Pterygota. The configuration of the odonatan axillary plate and the possibly intermediate state of the basalare of Ephemeroptera might even give some support to the hypothesis that Ephemeroptera and Odonata are sistergroups (i.e. Palaeoptera).

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Figure 1

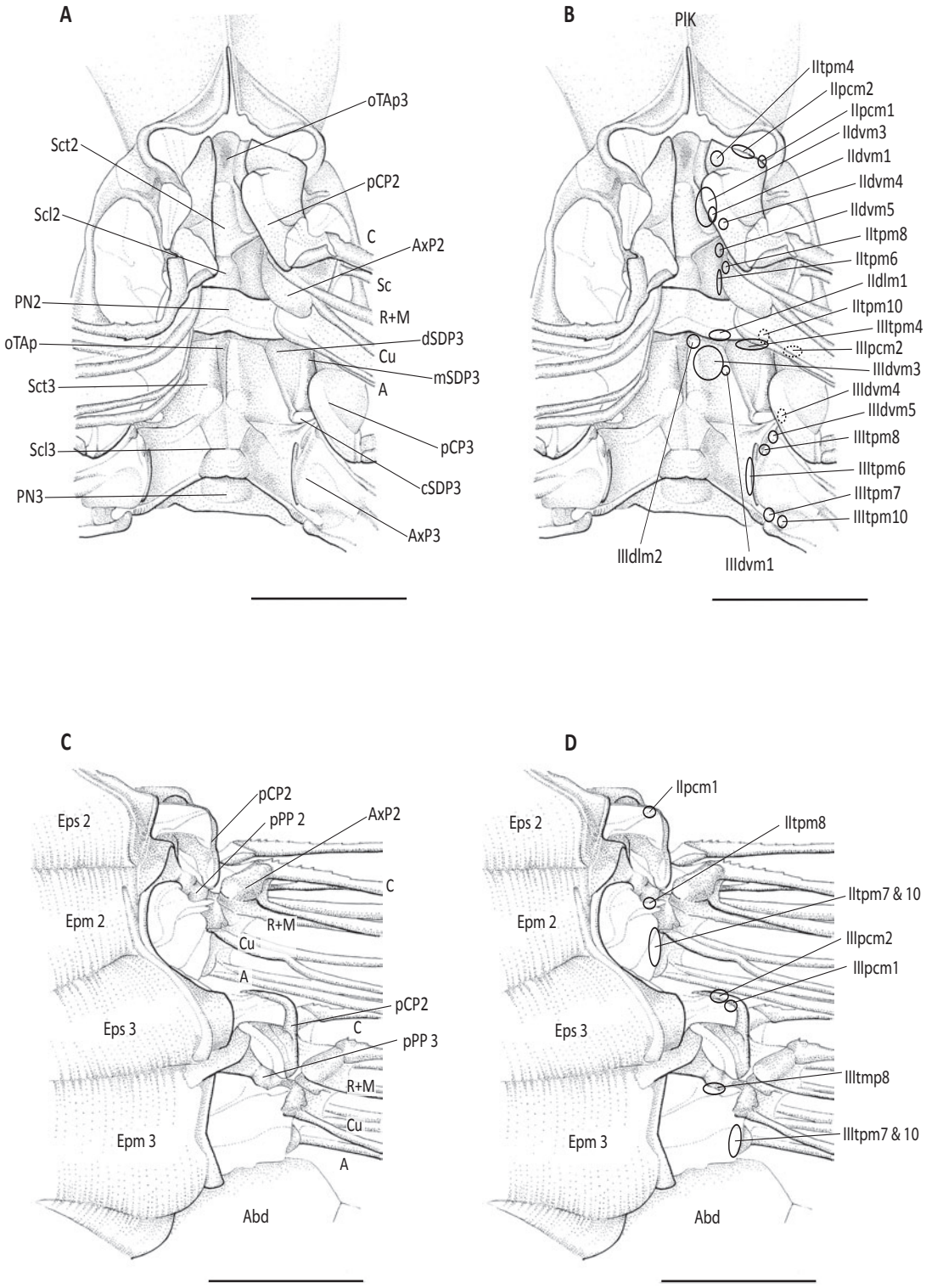


Figure 1: *Pyrroshoma nymphula*
A Tergum, scerites and veins.
B Tergum, muscle attachment points.
C Left pleurum, sclerites and veins.
D Left Pleurum, muscle attachment points.
Abbrevations: A - anal vein, Abd - abdominal segment, AxP - axillary plate, C - costa, cSDP - caudal semi-detached scutal plate, Cu - cubitus, dlm - dorso-longitudinal musculatur, dSDP - distal semi-detached scutal plate, dvm - dorso-ventral musculature, Epm - epimeron, Eps - episternum, M - media, mSDP - median semi-detached scutal plate, oTAp - origin of tergal apophyse, pcm - pleuro-coxal musculature, pCP - proximal costal plate, PlK - pleural keel, PN - postnotum, pPP - posterior pleural process, R - radius, Sc - subcosta, Scl - scutellum, Sct - scutum, tpm - tergo-pleural musculature,

Figure 2

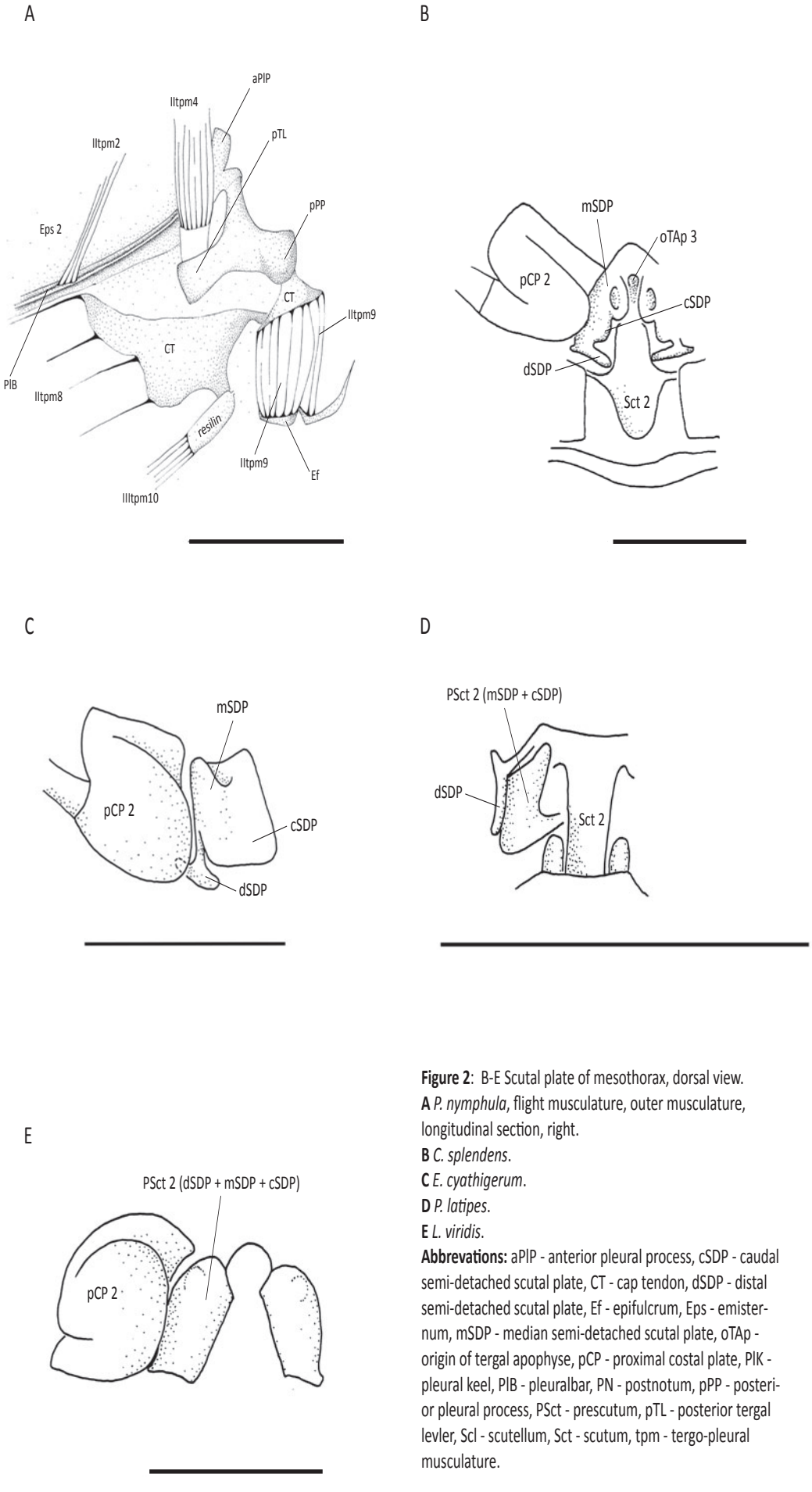
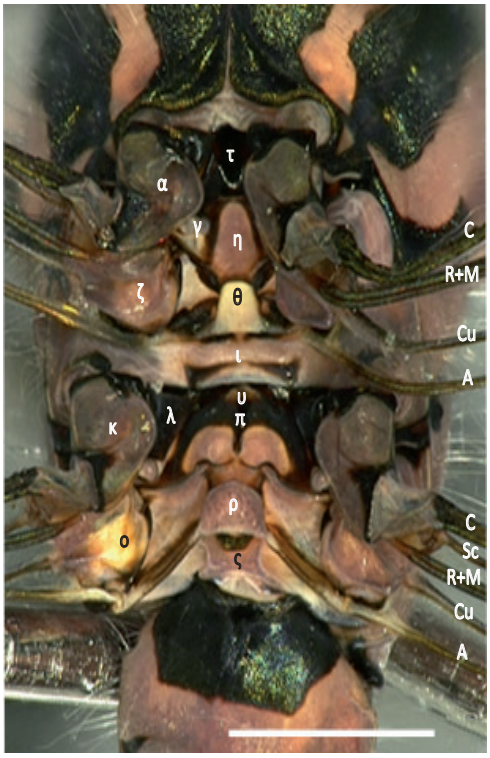
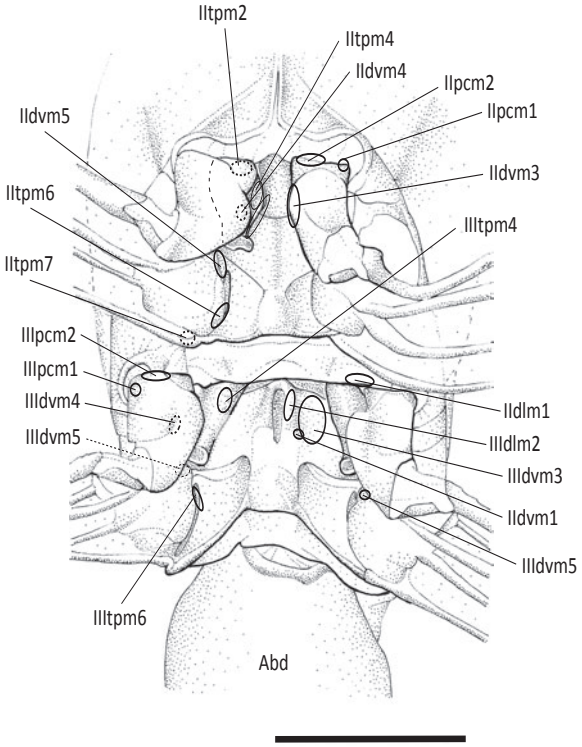


Figure 2: B-E Scutal plate of mesothorax, dorsal view.
A *P. nymphula*, flight musculature, outer musculature, longitudinal section, right.
B *C. splendens*.
C *E. cyathigerum*.
D *P. latipes*.
E *L. viridis*.
Abbrevations: aPlP - anterior pleural process, cSDP - caudal semi-detached scutal plate, CT - cap tendon, dSDP - distal semi-detached scutal plate, Ef - epifulcrum, Eps - emisternum, mSDP - median semi-detached scutal plate, oTAp - origin of tergal apophyse, pCP - proximal costal plate, PlK - pleural keel, PlB - pleuralbar, PN - postnotum, pPP - posterior pleural process, PSct - prescutum, pTL - posterior tergal levler, Scl - scutellum, Sct - scutum, tpm - tergo-pleural musculature.

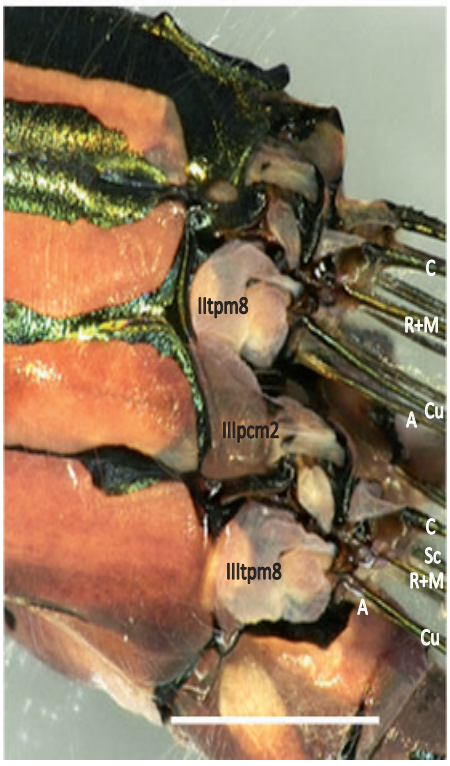
Supplement 1: *Coenagrion puella*



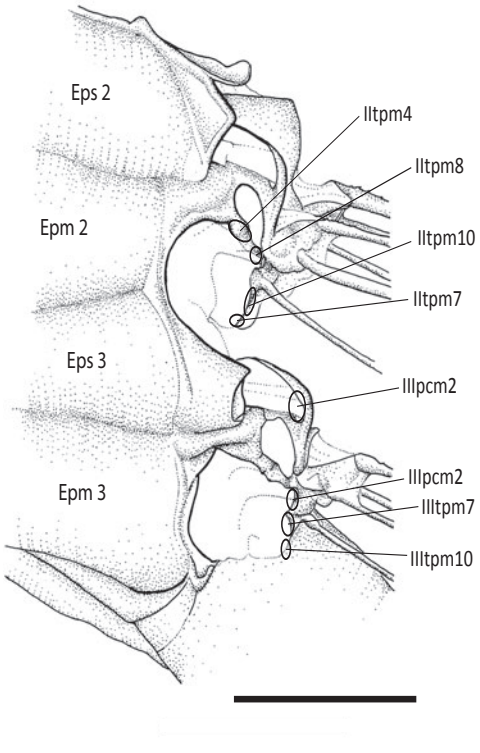
Tergum, sclerites and veins.



Tergum, muscle attachment points.



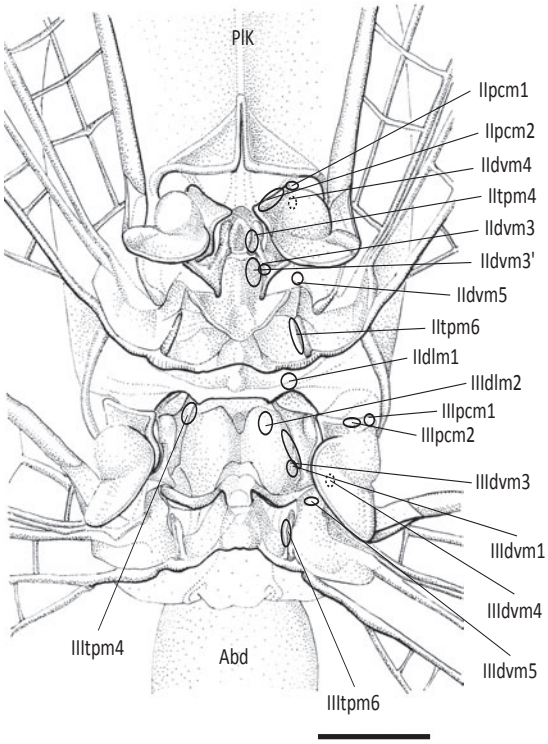
Left pleurum, muscles and veins.



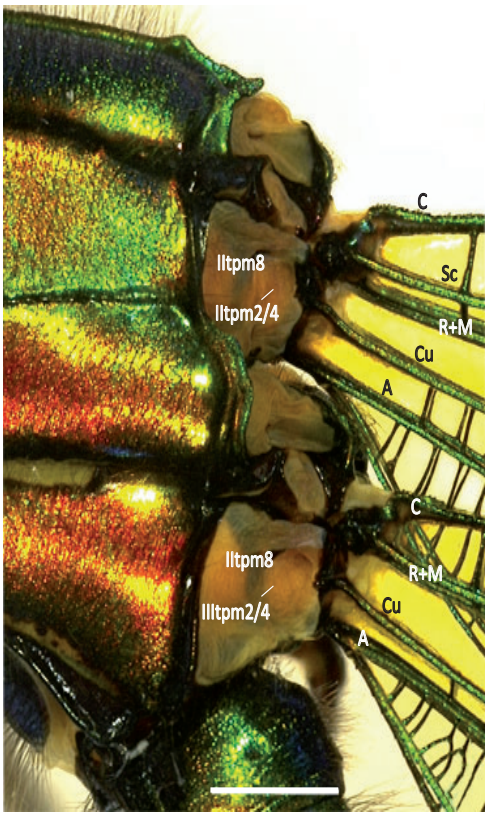
Left pleurum, muscle attachment points.

Sublement 2: *Calopteryx splendens*

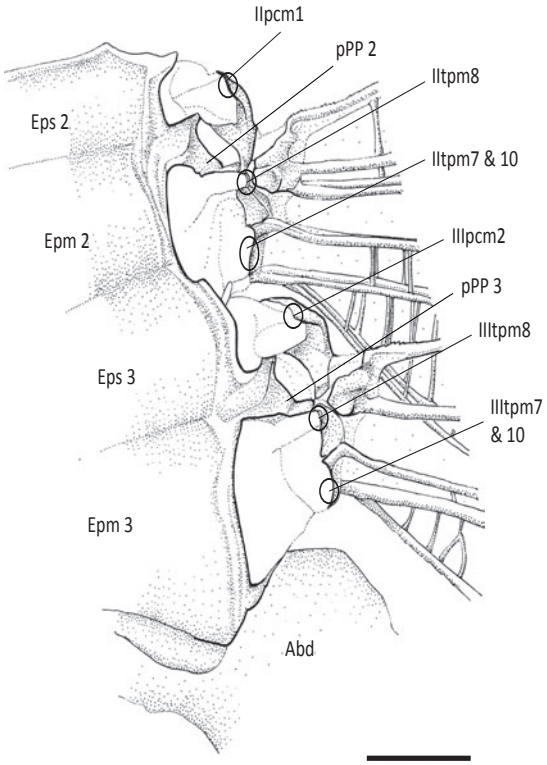
Tergum, sclerites and veins.



Tergum, msucle attachment points.

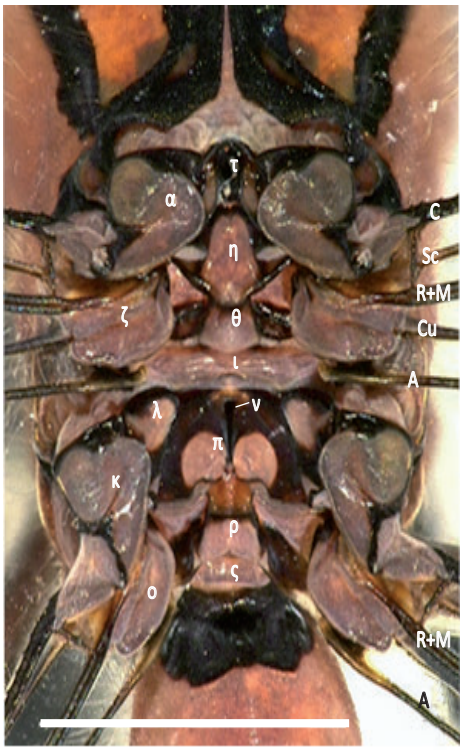


Left pleurum, muscles and veins.



Left pleurum, muscle attachment points.

Supplement 3: *Enallagma cyathigerum*



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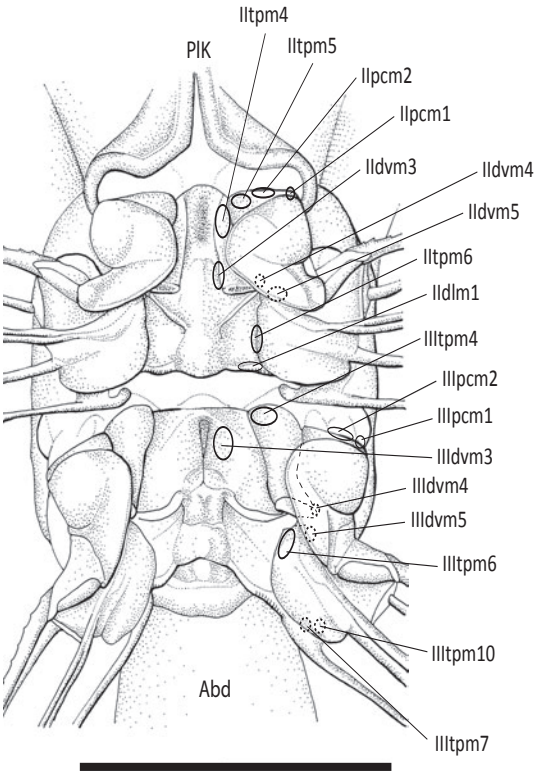
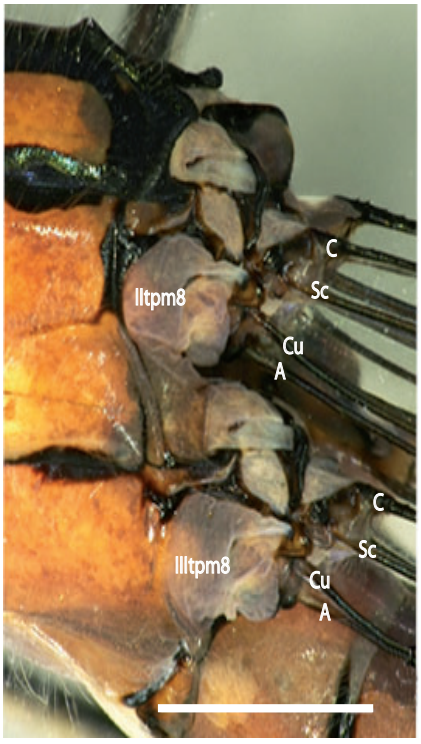
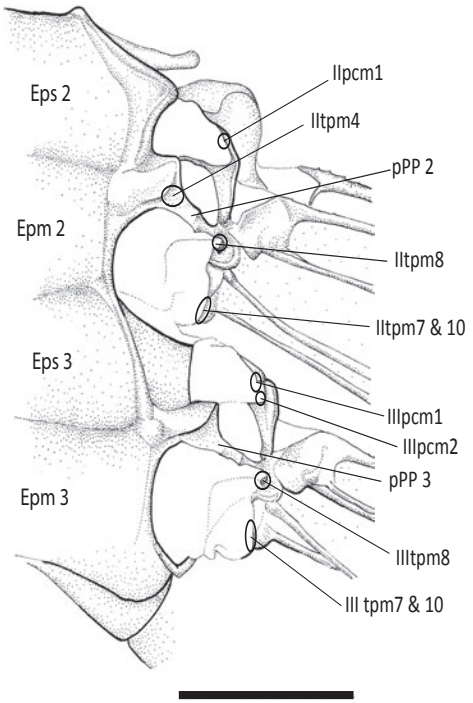


Tergum, muscle attachment points.



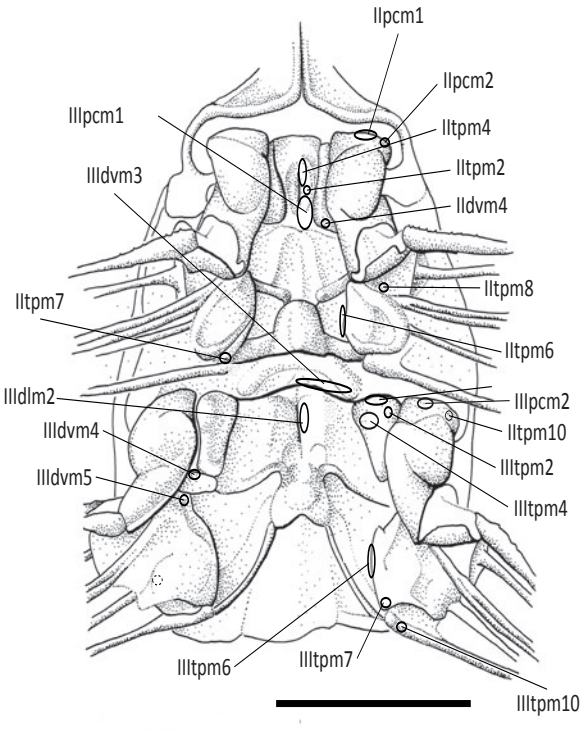
Left pleurum, muscles and veins.



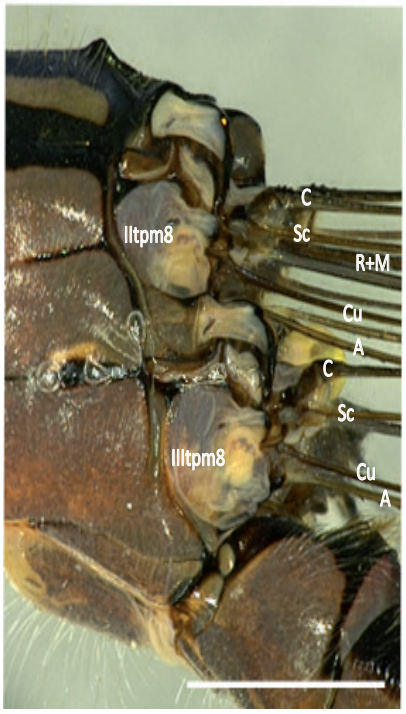
Left pleurum, muscle attachment points.

Supplement 4: *Ischnura elegans*

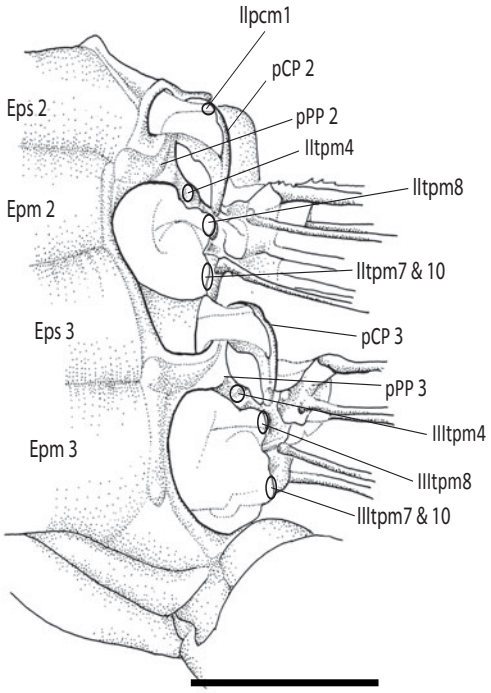
Tergum, sclerites and veins.



Tergum, muscle attachment points.

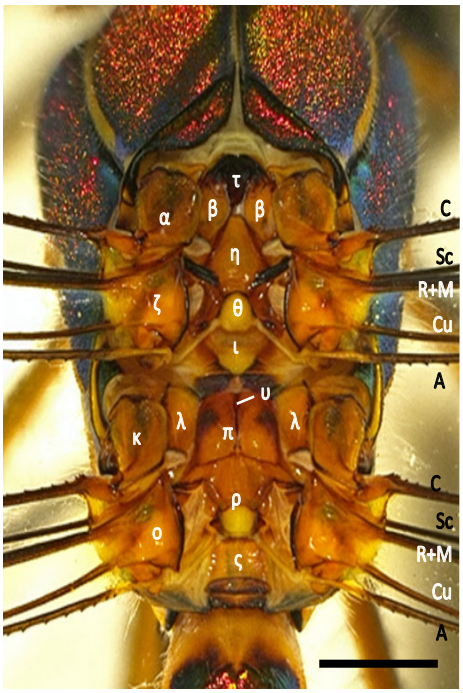


Left pleurum, muscles and veins.

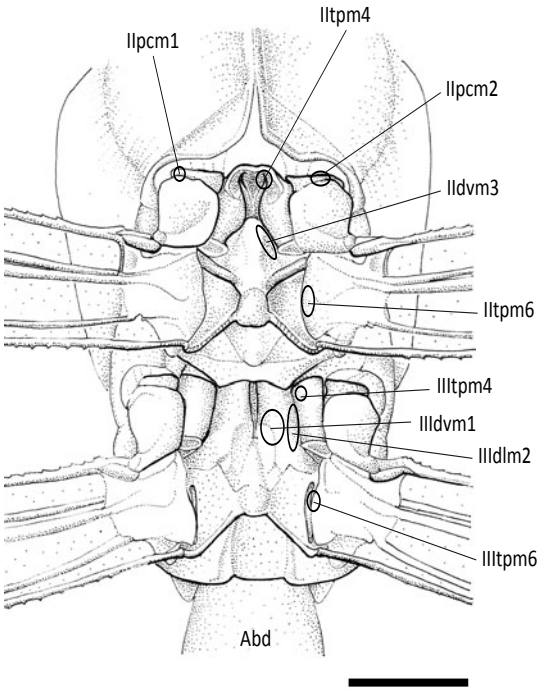


Left pleurum, msucle attachment points.

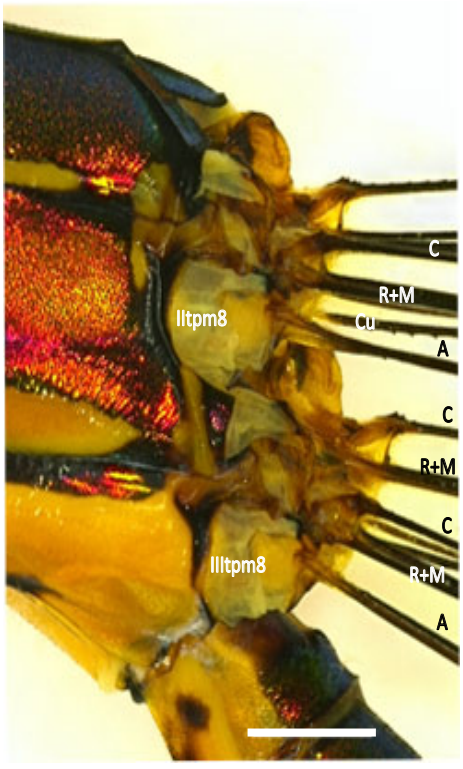
Supplement 2: *Lestes viridis*



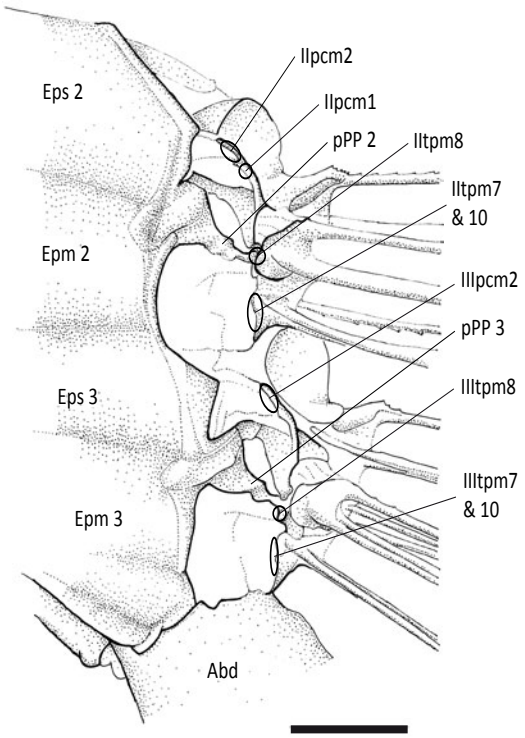
Tergum, sclerites and veins.



Tergum, muscle attachment points.

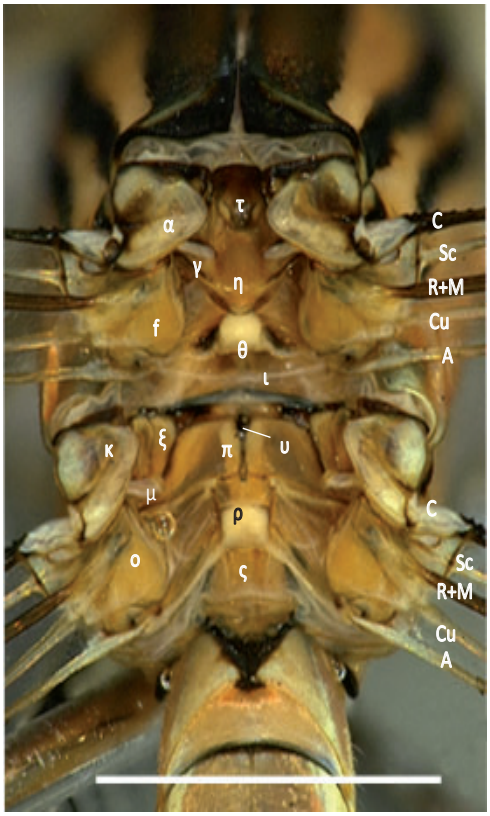


Left pleurum, muscle and veins.

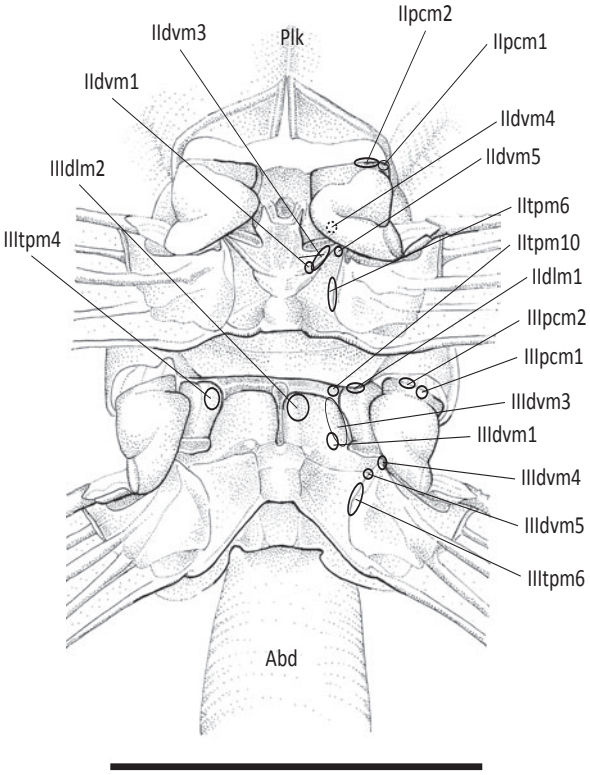


Left pleurum, muscle attachment points.

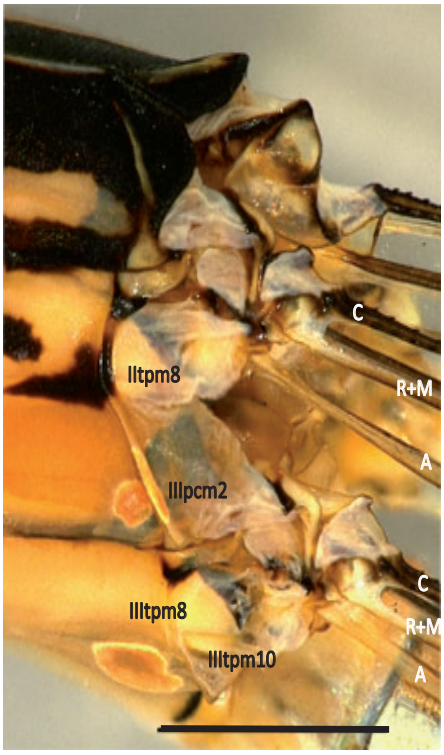
Supplement 6: *Platycnemides latipes*



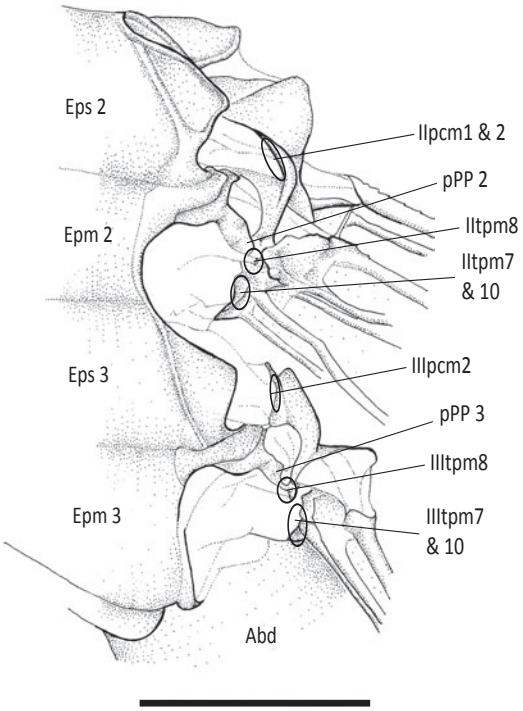
Tergum, sclerites and veins.



Tergum, muscle attachment points.

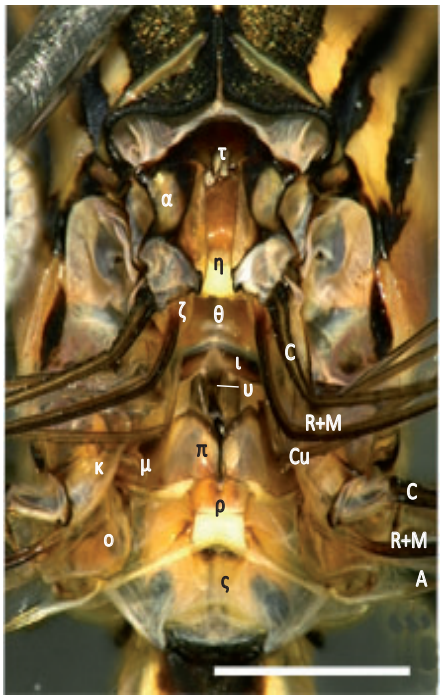


Left pleurum, muscles and veins.

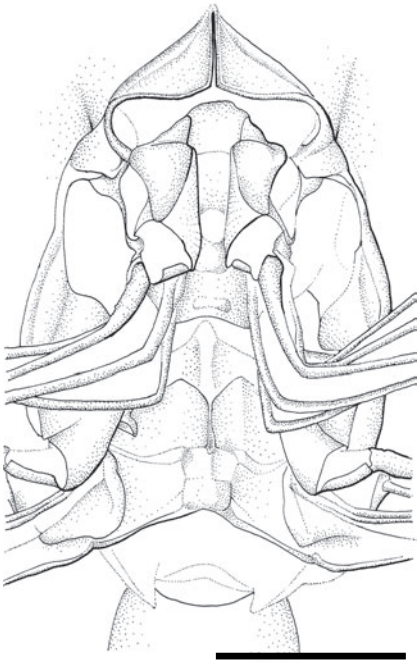


Left pleurum, muscle attachment points.

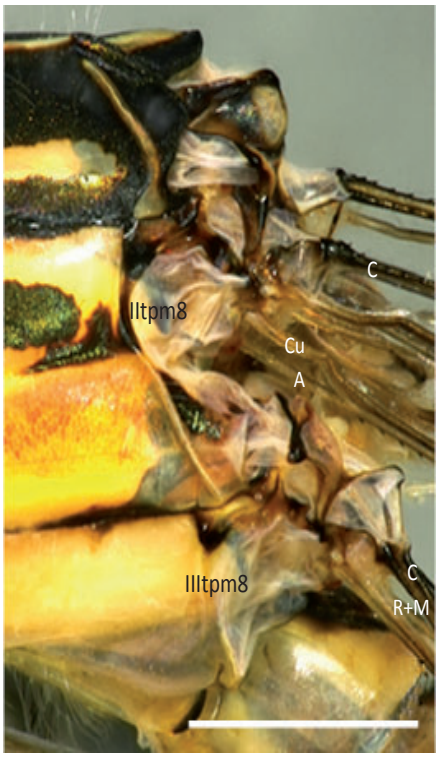
Supplement 7: *Platycnemides pennipes*



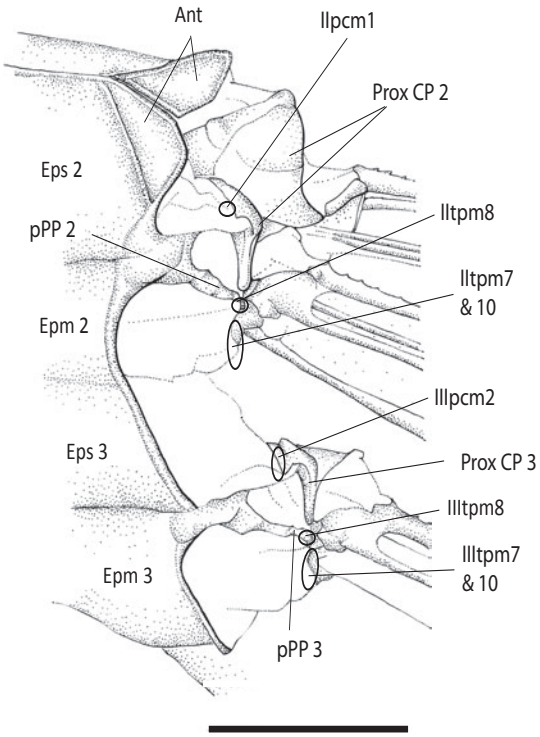
Tergum, sclerites and veins.



Tergum, without captions because of the data basis.



Left pleurum, muscles and veins.



Left pleurum, muscle attachment points.

Supplement 8: Abbreviations

A – anal vein
Abd – abdominal segment
AC - antecosta
Ant – antealar plate
aPlP - anterior pleural process
AxL – axillary ligament
AxP – axillary plate
C - costa
CAxP bridge - costa-axillary plate bridge
cSDP - caudal semi-detached scutal plate
CT – cap tendon
Cu - cubitus
Cx - coxa
dCP - distal costal plate
dSDP - distal semi-detached scutal plate
Ef - epifulcrum
Epm - epimeron
Eps - episternum
F - fulcrum
Fc - furca
FcB - furcalbranch
KEpm - katepimerom
KEps - katepisternum
M - media
mSDP - mediane semi-detached scutal plate
oTAp – origin of tergal apophyse
pCP - proximal costal plate
PCx - precoxa
PEps - preepisternum
PEpsAp – preepisternal apodem
PFc - prefurca
PlP – pleural process
PlK - pleural keel
PlB - pleuralbar
PN - postnotum
pPP – posterior pleural process
PSct - prescutum
pTL - posterior tergal levler
R - radius
Sc - subcosta
Scl - scutellum
Sct - scutum
TAp – tergal aphophyse

Additional abbreviations (supplemental figures)

α - proximale costal plate 2
β - prescutum 2
γ - caudal semi-detached scutal plate 2
δ - median semi-detached scutal plate 2
ε - distal semi-detached scutal plate 2
ζ – axillary plate 2
η - scutum 2
θ - scutellum 2
ι - postnotum 2
κ - proximale costal plate 2
λ - prescutum 3
μ - caudale semi-detached scutal plate 3
ν - mediane semi-detached scutal plate 3
ξ - distale semi-detached scutal plate 3
ο – axillary plate3
π - scutum 3
ρ - scutellum 3
ς - postnotum 3
τ – origin of apophyse 3
υ - origin of Apophyse 4

6. The Nymphal Thorax Musculature of Anisoptera (Insecta: Odonata) and Its Evolutionary Relevance

6.1. Contribution to this Manuscript

Conceived and designed the experiments: SB TH.
Performed the experiments: SB.
Analyzed the data: SB.
Contributed reagents/materials/analysis tools: SB TH.
Wrote the paper: SB TH.

6.2. Manuscript

Büsse S & Hörnschemeyer T (submitted) The nymphal thorax musculature of Anisoptera (Insecta: Odonata) and its evolutionary relevance. BMC Evolutionary Biology.

BMC Evolutionary Biology
Manuscript Draft

rMS ID : 1219094808101637

The thorax musculature of Anisoptera (Insecta: Odonata) nymphs and its evolutionary relevance.

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Abstract

Background: Among the winged insects (Pterygota) the Odonata (dragon- and damselflies) are special for several reasons. Their thorax morphology differs significantly from that of other Pterygota by a reduced number of muscles. Even within one individual, between the nymph and adult stage, the musculature is significantly different.
Results: Here we present a comparative morphological investigation of the thoracic musculature of dragonfly (Anisoptera) nymphs. For representatives of the Libellulidae, Aeshnidae and Cordulegasteridae we describe 71 muscles, 19 muscles of the prothorax, 26 muscles of the mesothorax and 27 muscles of the metathorax. This includes nine muscles that were so far unknown in Odonata, as well as seven muscles for which no homologous muscles could be identified in the neopteran thorax.
Conclusion: Our results support and extend the homology hypotheses considerable for the thoracic musculature of Odonata and Neoptera thus supplementing our understanding of the evolution of Pterygota and providing additional characters for phylogenetic analyses comprising all subgroups of Pterygota.

Keywords: Dragonflies, homology with Neoptera (Insecta), Odonata larvae, muscle setup

Background

The evolutionary diversification of highly functional structures can elucidate the adaption to special habitats and/or living conditions. The mostly direct flight muscle attachment in Odonata [e.g. 1-4] entails impressive flight skills and turns Odonata into the aerial key predators among the insects [5].

Beyond the Holometabola the Odonata are the insect group with the strongest variations between the habitat preferences of the nymphs and the adult. The adult is an elegant and skilled aerial predator whereas the larvae live and hunt in the water (e.g. 6, 5, 7].The ontogenetic development of the thoracic morphology of Odonata has nor been extensively studied so far. Poletaïew [8] reported that the wing buds occur at the 3rd or 4th nymph instar but the corresponding musculature is still undiscernibly. Maloeuf [9] remarked that the flight muscles in those instars are diminutive. He also stated that the Odonata nymphs have got a higher quantity and also stronger leg and neck muscles than the adults. The amount and the kind of muscles anyway significantly differ between the nymph and the adult Odonata [e.g. 9, 1]. During the ontogenesis the thoracic muscles are, in part, newly formed, transformed or reduced [10, 9]. The extend of these modifications seems to be exceptional in the Odonata if compared to other Pterygota, which sometimes exhibit an entire composition of musculature from the first instars onward [e.g. 11, 12].

The thorax musculature adult Anisoptera [e.g. 13, 2, 3] and also of *Epiophlebia superstes* Sélys, 1889 [1] is comparatively well-investigated. The thorax of adult Zygoptera has been studied comprehensively by Büsse et al. [4] and to some degree also by Asahina [1] and Ninomiya & Yoshizawa [14] whereas the thoracic musculature of the Odonata nymphs received little attention so far [10, 9, 1].

The insect flight is often considered as a key factor for the evolutionary success of the Pterygota [15]. Still, the origin and evolutionary development of the insect flight apparatus are unexplained. Furthermore, the motion transmission from the flight musculature to the wings is implemented in two different ways. The Odonata move their wings with a direct transmission whereas all other Pterygota got an indirect transmission [e.g. 16, 7]. Further differences in the flight apparatus appear in Ephemeroptera [e.g. 17, 12].

The relationships between the Odonata, Ephemeroptera and the Neoptera are still under discussion [18-21]. All three possible combinations of these three taxa were discussed: The sister-group relationship between Ephemeroptera and Odonata, called the Palaeoptera hypothesis, was already mentioned by Martynov [22]. He divided the Pterygota into two groups, the Palaeoptera, or “old winged” insects and the Neoptera, or “new winged” insects. This grouping is based mainly on the inability to fold their wings above the abdomen and the similarity of the wing base sclerites [22-31], or even on DNA analyses [32, 19]. Also the latest investigations of Blanke et al. [20, 21] and Thomas et al. [33] underline the Palaeoptera hypothesis. The second hypothetical sister-group relationship shows Odonata and Neoptera

closely related and is called Metapterygota hypothesis. With apomorphies like the lack of the ecdysis in the winged stage, the number and position of the mandible articulations and the therewith associated loss of several muscles, etc. [34-41, 15, 42, 12]. Also the DNA analysis of Ogden and Whiting [18] supports the Metapterygota hypothesis.
The third, the Chiastomyaria hypothesis, proposes a sister-group relationship of Ephemeroptera and Neoptera [43-45]. The strong dorso-longitudinal indirect wing depressor often considered as symplesiomorphic and the direct sperm transfer of the male to the female Gonoporus, which was often considered as convergent are the apomorphies of this weakest supported hypothesis [41]. However, a deeper understanding and a most widely accepted hypothesis is indispensable for elucidating the ground pattern of Pterygota and the understanding of the origin of insect flight [46].

One key to this phylogenetic topic and to the emergence and development of the insect flight might be the evolution of the flight apparatus of Odonata. To use this abundance of characters from the flight apparatus, for examples for phylogenetic analyses, the musculature of Odonata has to be homologized with the musculature of Neoptera.

Presently there seems to be widespread agreement on ground pattern hypotheses for the wing base sclerites and for the flight musculature in Neoptera [47, 12, 48, 49]. Even homologies between Ephemeroptera and Neoptera are mainly resolved [50, 48], while hypotheses on the homologies between Odonata and the remaining Pterygota are still under discussion [3, 50, 48, 14]; however, an approach for a solution has been presented recently [4].

In this comparative morphological analysis we study the musculature of the thorax of late nymphs from three groups of Anisoptera. These results identify variability among the Anisoptera and improve the understanding of the thorax musculature of Odonata. Furthermore, a homologization scheme for the thorax musculature with a generalized neopteran thorax [49], following and supplementing the study of Büsse et al. [4], is presented here. Our study allows new insights into the evolution of the odonatan thorax and may help to understand the early evolution of the insect flight apparatus.

Results

In the nymph thorax a total of 71 muscles were found: 19 muscles of the prothorax, 26 muscles of the mesothorax and 27 muscles of the metathorax. This includes nine muscles previously unknown for Odonata: Ispm1, IIscm1 (IIIscm1), IIscm2 (IIIscm2), IIscm8, IItpm3

(IIItpm3) and IIIscm4; as well as seven muscles, Ipcm9, Itpm7 – Itpm11, IIscm8 that have no homolog in the neopteran thorax.

The musculature of the thorax of *Sympetrum vulgatum* is used as a reference in the following descriptions. Characteristics of the other species were compared to *S. vulgatum* and differences were recorded.
We are aware that these results present a mixture of description and interpretation. However, stricter separation of these aspects would not support a clear and easily understandable presentation of the results. For establishing our homology hypotheses we supplemented our data with information from Maloeuf [9], Asahina [1], Ninomiya and Yoshizawa [14] and Büsse et al. [4], focusing on Asahina's [1] comprehensive study of *Epiophlebia superstes*. *E. superstes* represents a conspicuous mixture of anisopteran and zygopteran characters [1, 51, 7, 4]. Furthermore, in many aspects *Epiophlebia* seems to represent the most ancestral character distribution within the Odonata [e.g. 52].

For the skeletal elements of the thorax the nomenclature by Asahina [1] is used. Where necessary, this is supplemented by Snodgrass [53] and Ninomiya & Yoshizawa [14]. All muscles, including their attachment points, are listed as additional data (Additional file 1). Furthermore an additional table comparing our results with data from several other publications is also available as supporting information (Additional file 2).
For naming the thoracic muscles of Odonata the nomenclature of Friedrich & Beutel [49] and the homologies of Büsse et al. [4] were used where possible. Otherwise, a new name following the system of Friedrich & Beutel [49] was generated and marked with * in the following. The numbers in round brackets correspond to the nomenclature for Odonata from Asahina [1] and within those the numbers in round brackets are the homolog muscle of meso- and metathorax.

Musculature of the prothorax

dlm – Dorsal longitudinal muscles (Figure 1)

Idlm1 – Musculus prophragma-occipitalis (3)
Origin: Apex of tergal apophysis 2.
Insertion: Median at the postocciput.
Characteristics: Runs ventral to Idlm3. The point of origin in *A. affinis* is laterally at the caudal edge of tergite1.

ldlm3 – M. prophragma-cervicalis (2)
Origin: Tergal apophysis 1.
Insertion: Base of tergal apophysis 2.
Characteristics: Runs dorsal to ldlm1.

ldlm4 – M. cervico-occipitalis dorsalis (1)
Origin: Tergal apophysis 1.
Insertion: Median at postocciput.
Characteristics: Same direction as ldlm3. Comparatively small and short in *C. bidentatus*.

dvm – Dorsoventral muscles (Figure 2)

ldvm10 – M. profurca-phragmalis (20)
Origin: Apex of profurca.
Insertion: Apex of tergal apophysis 2.
Characteristics: Intersegmental muscle and homologous to IIIdvm8. Larval muscle [e.g. 1].

ldvm15 – M. propleuro-coxalis superior (13)
Origin: Antero-lateral part of tergite 1.
Insertion: Anterior procoxal rim.
Characteristics: Same point of insertion as ltpm9.

ldvm18 – M. pronto-coxalis lateralis (14&15)
Origin: Postero-lateral part of tergite 1.
Insertion: Lateral procoxal disk.
Characteristics: By far the largest muscle in the prothorax.

pcm – Pleuro-coxal muscles (Figure 3)

lpcm8 – M. propleuro-trochanteralis (18)
Origin: Dorso-median part of Episternum 1.
Insertion: Tendon of protrochanter.
Characteristics: Inserts at the same tendon as lpcm9 and lscm6.

lpcm9* – M. protergro- trochanteralis (17)
Origin: Lateral part of tergite 1, close to the pleura.
Insertion: Tendon of protrochanter.

Characteristics: Inserts at the same tendon as Ipcm8 and Iscm6, homolog to IIpcm5 and IIIpcm5 (see discussion).

scm – Sterno-coxal muscles (Figure 4)

Iscm2 – M. profurca-coxalis posterior (16)
Origin: External side of the base of profurca.
Insertion: Posterior procoxal rim.
Characteristics: Same point of origin as Iscm6.

Iscm6 – M. profurca-trochanteralis (19)
Origin: External side of the base of profurca.
Insertion: Tendon of protrochanter.
Characteristics: Inserts at the same tendon as Ipcm9 and Ipcm8.

spm – Sterno-pleural muscles (Figure 4)

Ispm1 – M. profurca-apodemalis (new for Odonata)
Origin: Apex of profurca.
Insertion: Apodem of propleura.
Characteristics: Homologous to the meso- and metathoracal muscles IIspm2 and IIIspm2.

tpm – Tergo-pleural muscles (Figure 5)

Itpm3 – M. pronoto-pleuralis anterior (12)
Origin: Lateral side of tergite 1.
Insertion: Anterior part of episternum 1.
Characteristics: Minute muscle.

Itpm7* – M. protergo-cervicalis posterior (4)
Origin: Lateral part of tergite 1.
Insertion: Lateral of cervix membrane.
Characteristics: Runs posterior to Itpm8.

Itpm8* – M. protergo-cervicalis anterior (5)
Origin: Most antero-lateral part of tergite 1.
Insertion: Lateral of cervix membrane.

Characteristics: Runs anterior to ltpm7. Most antero-lateral muscle in the prothorax; in *A. affinis* the point of insertion is very close to the boundary of the caput.

ltpm9* – M. protergo-preepisternalis (7)
Origin: Tergite 1, lateral of tergal apophysis 2.
Insertion: Base of preepisternal apodem 1.
Characteristics: Origin is located postero-lateral of ltpm7 and ltpm8.

ltpm10* – M. prosterna-coxalis dextra (9)
Origin: Apex of right preepisternal apodem 1.
Insertion: Anterior of left procoxal rim.
Characteristics: Intersects with muscle ltpm11, more or less in the middle of the anterior part of the prothorax.

ltpm11* – M. prosterna-coxalis sinister (10)
Origin: Apex of left preepisternal apodem 1.
Insertion: Anterior of right procoxal rim.
Characteristics: Intersects with muscle ltpm10, more or less in the middle of the anterior part of the prothorax.

vlm – Ventral longitudinal muscles (Figure 1)

lvlm3 – M. profurca-tentorialis (11)
Origin: Apex of profurca.
Insertion: Cranial at the tentorial bar.
Characteristics: Runs into the head capsule.

lvlm7 – M. profurca-mesofurcalis (41)
Origin: Anterior part of furca-branch 2.
Insertion: Posterior part of furca 1.
Characteristics: In Asahina [1] a muscle of the mesothorax.

Musculature of the pterothorax

The meso- and metathorax of Odonata is coalesced into a funcional unit, the synthorax [54]. The thorax of anisopteran larvae is less sclerotized in the pleurotergal region than it is in the

adults. In this part of the thorax the wings develop through all instars [55].

Musculature of the mesothorax

dlm – Dorsal longitudinal muscles (Figure 1)

IIdlm1 (25 (45)) – M. prophragma-mesophragmalis
Origin: Tergal apophysis 3.
Insertion: Tergal aposphysis 4.
Characteristics: Minute muscle.

dvm – Dorsoventral muscles (Figure 2)

IIdvm1- M. mesonoto-sternalis (23‘ (46‘))
Origin: Base of mesofurca.
Insertion: Postero-lateral edge of mesothoracic wing bud.
Characteristics: Inserted distal of IIdvm3 and not identifiable in *S. vulgatum*.

IIdvm3- M. mesonoto-trochantinalis posterior (23 (46))
Origin: Base of mesofurca.
Insertion: Antero-lateral edge of mesothoracic wing bud.
Characteristics: Same point of insertion as IIdvm4 and IIdvm5.

IIdvm4 - M. mesonoto-coxalis anterior (26 (48))
Origin: Anterio-lateral edge of mesocoxa.
Insertion: Antero-lateral edge of mesothoracic wing bud.
Characteristics: Same point of insertion as IIdvm3 and IIdvm5.

IIdvm5 - M. mesonoto-coxalis posterior (27 (49))
Origin: Lateral at the mesocoxal disk.
Insertion: Antero-lateral edge of mesothoracic wing bud.
Characteristics: Same point of insertion as IIdvm3 and IIdvm4. Single-branched in *S. vulgatum* [cf. 1]. In *A. affinis* and *C. bidentatus* a dichotomous muscle [cf. 9].

IIdvm6 - M. mesocoxa-subalaris (37 (60))

Characteristics: Strongest muscle in the mesothorax.

pcm – Pleuro-coxal muscles (Figure 3)

IIpcm1 - M. mesanepisterno-trochantinalis (21 (43))
Origin: Preepisternum 2, close to the intersegment boarder.
Insertion: Lateral of the tergal apophysis 2 at tergite 2.
Characteristics: Same point of insertion as IIpcm2, it is the strongest muscle in the mesothorax of *A. affinis* and *C. bidentatus*.

IIpcm2 - M. mesobasalare-trochantinalis (22 (44))
Origin: Base of preepisternal apodem 2.
Insertion: Lateral of the tergal apophysis 2 at tergite 2.
Characteristics: Same point of insertion as IIpcm1; minute muscle.

IIpcm4 - M. mesanepisterno-coxalis posterior (36 (58))
Origin: At the base of the interpleural ridge 2.
Insertion: Antero-external part of mesocoxa.
Characteristics: The muscle runs far laterally – connected very close to the pleuron.

IIpcm6 - M. mesopleura-trochanteralis (39 (62))
Origin: Dorsal part of katepisternum 2.
Insertion: Tendon of mesotrochanter.
Characteristics: Same tendon as IIscm6.

scm – Sterno-coxal muscles (Figure 4)

IIscm1 - M. mesofurca-coxalis anterior (new for Odonata)
Origin: Lateral base of mesofurca.
Insertion: Antero-external ridge of mesocoxa.
Characteristics: Postero-lateral of IIpcm4.

IIscm2 - M. mesofurca-coxalis posterior (new for Odonata)
Origin: Lowermost part of mesofurca.
Insertion: Postero-lateral apodem of mesocoxa.
Characteristics: Point of origin is ventral to IIscm6 and point of insertion ventral to IIscm3.

IIscm3 - M. mesofurca-coxalis medialis (38 (61))
Origin: Lateral base of mesofurca.
Insertion: Postero-lateral apodem of mesocoxa.
Characteristics: Runs medially to IIdvm6.

IIscm6 - M. mesofurca-trochanteralis (40 (63))
Origin: Latero-external side of mesofurca.
Insertion: Tendon of mesotrochanter.
Characteristics: Same tendon as IIpcm6.

IIscm7 - M. mesospina-metacoxalis (59)
Origin: Preepisternal apodem.
Insertion: Antero-lateral edge of metacoxa.
Characteristics: Intersegmental muscle. Asahina [1] described this muscle as a larval muscle of the metathorax.

IIscm8* - M. mesospina-mesocoxalis (new for Odonata)
Origin: Medio-ventral part of preepisternal apodem.
Insertion: Postero-lateral at mesocoxa, close to IIdvm6.
Characteristics: Funnel-shaped muscle.

spm – Sterno-pleural muscles (Figure 4)

IIspm2 – M. mesofurca-pleuralis (35 (57))
Origin: Apex of mesofurca.
Insert: Interpleural ridge 2.
Characteristics: Larval muscle [e.g. 1].

tpm – Tergo-pleural muscles (Figure 5)

IItpm3 – M. mesonoto-basalaris (new for Odonata)
Origin: Dorsal side of mesothoracic wing bud, anterior to the origin of IItpm4.
Insertion: Ventral side of mesothoracic wing bud, anterior to origin of IItpm4.
Characteristics: Runs within the wing bud, anterior to IItpm4.

IItpm4 – M. mesonoto-pleuralis anterior (28 (50))
Origin: Dorsal side of mesothoracic wing bud, posterior to the origin of IItpm3.

Insertion: Ventral side of mesothoracic wing bud, posterior to the origin of IItpm3.
Characteristics: Runs within the wing bud, posterior to IItpm3.

IItpm6 - M. mesonoto-pleuralis posterior (31 (53))
Origin: Upper part of interpleural ridge 2.
Insertion: Antero-dorsal edge of mesothoracic wing bud.

IItpm7 - M. mesanepisterno-axillaris (33 (55))
Origin: Ventral part of epimeron 2.
Insertion: Lateral edge of mesothoracic wing bud.
Characteristics: Runs between IItpm8 (anterior) and IItpm10 (posterior)

IItpm8 - M. mesepimero-axillaris secundus (32 (54))
Origin: Ventral part of epimeron 2.
Insertion: Lateral edge of mesothoracic wing bud.
Characteristics: Anterior to IItpm8 and IItpm10.

IItpm9 – M. mesepimero-axillaris tertius (29/30 (51/52))
Origin: Dorsal part of epimeron 2.
Insertion: Inner side of ventral part of mesothoracic wing bud.
Characteristics: Only one muscle is recognizable (see discussion)

IItpm10 – M. mesepimero-subalaris (34 (56))
Origin: At the base of the interpleural ridge 2.
Insertion: Lateral edge of mesothoracic wing bud.
Characteristics: Posterior to IItpm7 and IItpm8.

vlm – Ventral longitudinal muscles (Figure 1)

IIvlm6 - M. mesospina-abdominosternalis (68)
Origin: Posterior part of preepisternal apodem 3.
Insertion: Antecostal apodem [e.g. 9].
Characteristics: This muscle runs from the mesothorax into the abdomen.

IIvlm7 - M. mesofurca-abdominosternalis (42 (64))
Origin: Posterior at the mesofurca.
Insertion: Within the abdomen, in Asahina [1] anterior margin of first abdominal sternite.

Characteristics: Runs from the mesothorax through the metathorax into the abdomen.

Muscles of the metathorax

dlm – Dorsal longitudinal muscles (Figure 1)

IIIdlm1 - M. mesophragma-metaphragmalis (45(25))
Origin: Tergal apophysis 4.
Insertion: Transversal ridge between abdomen and thorax, sternal part.
Characteristics: Dorso-ventral of IIIdlm2.

IIIdlm2 - M. metanoto-phragmalis (45‘)
Origin: Tergal apophysis 4.
Insertion: Transversal ridge between abdomen and thorax, sternal part.
Characteristics: Ventro-lateral of IIIdvm1.

dvm – Dorsoventral muscles (Figure 2)

IIIdvm1- M. mesonoto-sternalis (46‘ (23‘))
Origin: Base of metafurca.
Insertion: Postero-lateral edge of metathoracic wing bud.
Characteristics: Inserted distal of IIIdvm3. Not present or identifiable in *S. vulgatum*.

IIIdvm3 - M. metanoto-trochantinalis (46 (23))
Origin: Base of metafurca.
Insertion: Antero-lateral edge of metathoracic wing bud.
Characteristics: Same point of insertion as IIIdvm4 and IIIdvm5.

IIIdvm4 - M. metanoto-coxalis anterior (48 (26))
Origin: Anterio-lateral edge of metaocoxa.
Insertion: Antero-lateral edge of metathoracic wing bud.
Characteristics: Same point of insertion as IIIdvm3 and IIIdvm5.

IIIdvm5 - M. metanoto-coxalis posterior (49 (27))
Origin: Median part of metacoxaldisk.
Insertion: Antero-lateral edge of metathoracic wing bud.

Characteristics: Same point of insertion as IIIdvm3 and IIIdvm4. It is a single-branched muscle, not like the homologous mesothoracic muscle IIdvm5 [e.g. 9, 1].

IIdvm6 - M. metacoxa-subalaris (60(37))
Origin: Lateral part of tergite 3.
Insertion: Postero-lateral part of metacoxa.
Characteristics: Strongest muscle in the metathorax.

IIIdvm8 - M. metanoto-phragmalis (67)
Origin: Dorsal part of the posterior ridge of epimeron 3.
Insertion: Posterior end of metafurca.

pcm – Pleuro-coxal muscles (Figure 3)

IIIpcm1 - M. metanepisterno-trochantinalis (43 (21))
Origin: Preepisternum 3.
Insertion: Lateral of the tergal apophysis 3 at tergite 3.
Characteristics: Same point of insertion as IIIpcm2. AThis is the strongest muscle in the metathorax of *A. affinis* and *C. bidentatus*.

IIIpcm2 - M. metabasalare-trochantinalis (44(22))
Origin: Base of preepisternal apodem 3.
Insertion: Lateral of the tergal apophysis 3 at tergite 3.
Characteristics: Minute muscle with the same point of insertion as IIpcm1.

IIIpcm4 - M. metanepisterno-coxalis posterior (58 (36))
Origin: Dorsal part of interpleuralridge 3.
Insertion: Antero-external part of metacoxa.
Characteristics: The muscle runs laterally.

IIIpcm6 - M. mesopleura-trochanteralis (39 (62))
Origin: Dorsal part of katepisternum 3.
Insertion: Tendon of metatrochanter.
Characteristics: Same tendon as IIIscm6. In *A. affinis* and *C. bidentatus*.the origin nearly covers the whole katepisternum in *A. affinis* and *C. bidentatus*.

scm – Sterno-coxal muscles (Figure 4)

IIIscm1 - M. metafurca-coxalis anterior (new for Odonata)
Origin: Lateral base of metafurca.
Insertion: Antero-external ridge of metacoxa.
Charecteristics: Postero-lateral of IIIpcm4.

IIIscm2 - M. metafurca-coxalis posterior (new for Odonata)
Origin: Lowermost part of metafurca.
Insertion: Postero-lateral apodem of metacoxa.
Charecteristics: Point of origin is ventral to IIIscm6 and point of insertion ventral to IIIscm3.

IIIscm3 - M. metafurca-coxalis medialis (61(38))
Origin: Lateral base of metafurca.
Insertion: Postero-lateral apodem of metacoxa.
Characteristics: Runs medially to IIIdvm6.

IIIscm4 - M. metafurca-coxalis lateralis (new for Odonata)
Origin: Apex of metafurca.
Insertion: Lateral base of metacoxa, at the border of pleurite
Characteristics: Very thin and lateral running muscle.

IIIscm6 - M. metafurca-trochanteralis (63 (40))
Origin: Latero-external side of metafurca.
Insertion: Tendon of metatrochanter.
Characteristics: Same tendon as IIIpcm6.

spm – Sterno-pleural muscles (Figure 4)

IIIspm2 – M. metafurca-pleuralis (57 (35))
Origin: Apex of metafurca.
Insert: Median part of interpleural ridge 3.
Characteristics: Larval muscle [e.g. 1].

tpm – Tergo-pleural muscles (Figure 5)

IIItpm3 – M. metanoto-basalaris (new for Odonata)
Origin: Dorsal side of metathoracic wing bud, anterior to the origin of IIItpm4.

Insertion: Ventral side of metathoracic wing bud, anterior to origin of IIItpm4.
Characteristics: Runs within the wing bud, anterior to IIItpm4.

IIItpm4 – M. metanoto-pleuralis anterior (50 (28))
Origin: Dorsal side of metathoracic wing bud, posterior to the origin of IIItpm3.
Insertion: Ventral side of metathoracic wing bud, posterior to the origin of IIItpm3.
Characteristics: Runs within the wing bud, posterior to IIItpm3

IIItpm6 - M. metanoto-pleuralis posterior (53 (31))
Origin: Upper part of interpleural ridge 3.
Insertion: Antero-dorsal edge of metathoracic wing bud.

IIItpm7 - M. metanepisterno-axillaris (55 (33))
Origin: Ventral part of epimeron 3.
Insertion: Lateral edge of metathoracic wing bud.
Characteristics: Runs between IIItpm8 (anterior) and IIItpm10 (posterior).

IIItpm8 - M. metapimero-axillaris secundus (54 (32))
Origin: Ventral part of epimeron 3.
Insertion: Lateral edge of metathoracic wing bud.
Characteristics: Anterior to IIItpm8 and IIItpm10.

IIItpm9 – M. metapimero-axillaris tertius (51/52 (29/30))
Origin: Dorsal part of epimeron 3.
Insertion: Inner side of ventral part of metathoracic wing bud.
Characteristics: Only one muscle is recognizable (see discussion).

IIItpm10 – M. metapimero-subalaris (56 (34))
Origin: Median part of interpleural ridge 3.
Insertion: Lateral edge of metathoracic wing bud.
Characteristics: Posterior to IIItpm7 and IIItpm8.

vlm – Ventral longitudinal muscles (Figure 1)

IIIvlm2 - M. mesofurca-abdominosternalis (65)

Characteristics: Not identifiable in *S. vulgatum*.

IIIvlm3 - M. metaspina-abdominosternalis (66)
Origin: Posterior part of sternum 3.
Insertion: Within the abdomen, second abdominal sternite.
Characteristics: This muscle is caudal distinctly flattened.

Discussion

Musculature of the prothorax

dlm – Dorsal longitudinal muscles (Figure 1)

The tergal apophyses of Odonata are not homologous to the primary phragmata [56], but to the pseudo phragmata [54]. Therefore, the homology of Idlm1, Idlm3 and Idlm4 is unquestionable [cf. 49].

dvm – Dorsoventral muscles (Figure 2)

The muscle Idvm10 is only present in odonatan nymphs [9, 1]. Because of the correspondence of the pseudo phragmata with the tergal apophysis the assuming a homology with the neopteran muscle is straightforward. The same is true for the homology of Idvm15 and Idvm18, because of the identical attachment points [cf. 49]. Maloeuf [9] and Asahina [1] described Idvm18 as two muscles M14 and an independent nymph muscle M15. This muscle is rather separated in bundles, which belong to one muscle, at least in Anisoptera. This muscle is very strong in nymphs and the pleural part of this coxa remoter [9] seems to be unique in nymphs. It assumes a reinforcing effect, which may be necessary for adaption to the walking lifestyle of the nymphs.

pcm – Pleuro-coxal muscles (Figure 3)

The points of origin of Ipcm8 and Ipcm9, at the episternum 1 and the lateral part of tergite 1, are slightly relocated in comparison to Epiophlebia where Asahina [1] described them at the epimeron 1 and the median lobe of tergite 1, respectively. However, the neopteran homolog of Ipcm8 has similar attachment points [cf. 49]. Muscle Ipcm9 has no homologous muscle in the generalized Neoptera thorax [49]. It seems to represents a unique odonatan muscle. The muscle Ipcm9 was interpreted as pleural muscles, even though its origin is on the tergite,

because of the positions of its homologous muscles IIpcm5 and IIIpcm5 in the mesothorax and metathorax [cf. 49]. This seems to be an interesting evolutionary trade-off, because IIpcm5 and IIIpcm5 are not present in Odonata. The pterygote ground pattern might show Ipcm9, IIpcm5 and IIIpcm5 as homolog muscles in pro-, meso- and metathorax. During the evolution of Odonata IIpcm5 and IIIpcm5 were reduced, wheras Ipcm9 was reduced during the evolution of the Neoptera.

scm – Sterno-coxal muscles (Figure 4)

The attachment points of Iscm2 and Iscm6 are congruent with *Epiophlebia* [1] as well as their neopteran counterparts [cf. 49].

spm – Sterno-pleural muscles (Figure 4)

The muscle Ispm1 is new for Odonata neither Asahina [1] nor Maloeuf [9] described it. The attachment points coincide with those of its neopteran homolog [cf. 49].

tpm – Tergo-pleural muscles (Figure 5)

Itpm3 has been homologized with its neopteran counterpart; because it has the same attachment points [cf. 49]. The muscles Itpm7, Itpm8, Itpm9, Itpm10 and Itpm11 show no counterpart in the thorax of Neoptera. Some of the attachment points of the muscles Itpm7-Itpm11 differ slightly from the descriptions of Maloeuf [9] and Asahina [1].

vlm – Ventral longitudinal muscles (Figure 1)

Asahina [1] described Ivlm7 under the name M41 as a muscle of the mesothorax, whereas Maloeuf [9] named it M42 in the prothorax.

Musculature of the pterothorax

dlm – Dorsal longitudinal muscles (Figure 1)

As in the prothorax the homology of IIdlm1 (IIIdlm1) is unequivocal [cf. 49] because of the position of its attachment points on the tergal. Even though IIIdlm2 has no homolog in the mesothorax, its attachment points are congruent with those of its counterpart in the Neoptera, which supports our homology interpretation. According to Büsse et al. [4] muscle IIIdlm2 is

only present in Zygoptera and *Epiophlebia*; this assumption can be refuted, at least for the nymphs.

dvm – Dorsoventral muscles (Figure 2)

The attachment points of IIdvm4 (IIIdvm4) and IIdvm5 (IIIdvm5) as well as the points of insertion of IIdvm1 (IIIdvm1) and IIdvm3 (IIIdvm3) are identical [49], because the wing buds are a subset of the notum and were relocated during the development to the adult. During metamorphosis the wing bud turn upside down. At this, the dorsal part of the wing buds represent the tergal structures especially the wing base sclerites, whereas, the ventral part of the wing bud represent the lateral regions of the notum and the most dorsal area of the pleuron in the adult. The points of origin and insertion of the adult zygopteran dorso-ventral muscles are usually shifted to some degree in comparison to Neoptera [4], because of the changes in shape and size of the notum in Odonata. The points of origin of IIdvm1 (IIIdvm1), IIdvm3 (IIIdvm3) and IIdvm6 (IIIdvm6) differ slightly from Neoptera [cf. 49] but these relocations do not affect the function of these muscles as elevators of the wings [4].
Muscle IIdvm1 (IIIdvm1) is not identifiable in *S. vulgatum*, but distinct in *A. affinis* and *C. bidentatus*. Muscle IIdvm5 is single-branched in *S. vulgatum* [like in Asahina 1954] and dichotomous in *A. affinis* and *C. bidentatus* [cf. 9]. Muscle IIIdvm5 is single-branched in all species studied.
Muscle IIIdvm8 has no homolog in the mesothorax; the insertion is identical to the Neoptera [cf. 49]. The point of origin at the edge of the epimeron 3 corresponds to the neopteran metaphragma [53].

pcm – Pleuro-coxal muscles (Figure 3)

The points of origin of IIpcm4 (IIIpcm4) and IIpcm6 (IIIpcm6) at the pleurum differ slightly. In muscles IIpcm1 and IIpcm2 (IIIpcm2) the functional attachment point (point of insertion) slightly relocated dorsally from the mesobasalare, respectively the mesanepisternum to the tergalapophysis 2 due to their function as direct flight muscles [9, 4]. These structures are not homologous, but the function as flight muscles is retained and due to the parsimony principle a homology is likely.

Muscle IIpcm1 (IIIpcm1) is the strongest muscle in the mesothorax of *A. affinis* and *C. bidentatus*, whereas, in *S. vulgatum* it is IIdvm6. Furthermore, muscle IIpcm1 (IIIpcm1) was described as M21 (M43) by Asahina [1] and as M22 (M44) by Maloeuf [9]. Muscle IIvpcm2 (IIIpcm2) is M22 (M44) in Asahina [1] and M21 (M43) in Maloeuf [9].

scm – Sterno-coxal muscles (Figure 4)

The muscles IIscm1 (IIIscm1), IIscm2 (IIIscm2) and IIscm8 are new for Odonata, neither Maloeuf [9], Asahina [1] nor Büsse et al. [4] mentioned them. The muscles IIscm1 (IIIscm1) and IIscm2 (IIIscm2) are not present in adult Zygoptera [4]. Therefore, they could represent either unique muscles for nymph Odonata or unique muscles for Anisoptera (or Epiprocta); even a combination is conceivable. However, the homology of IIscm1, IIscm2, IIscm3, IIscm6 and IIscm7 is well supported by their positions in relation to other structures in the thorax. Muscle IIscm8 is not present in the generalized neopteran thorax [cf. 49]. Furthermore, this muscle has no counterpart in the pro- or metathorax. Asahina [1] described muscle IIscm7 as a nymph muscle. Our results and its lacking in adult Zygoptera [4] confirm this interpretation. Muscle IIscm7 has no homologous muscle in the metathorax. Muscle IIIscm4 has no homolog in the mesothorax.

spm – Sterno-pleural muscles (Figure 4)

Asahina's [1] interpretation of muscle IIspm2 (IIIspm2) as a nymph muscle is supported by our results and the lack in adult Zygoptera [4].

tpm – Tergo-pleural muscles (Figure 5)

Muscle IItpm3 (IIItpm3) is new for Odonata. All tergo-pleural muscles have at least one attachment point within the wing bud. Nevertheless, the homologization are quite straightforward, because of the upside down turning of the wing buds during metamorphosis (see also dvm above). The points of origin in muscle IItpm6 (IIItpm6) – IItpm10 (IIItpm10) are slightly relocated, for example muscle IItpm6 originates at the interpleural ridge in Odonata and at the pleural arm in Neoptera [cf. 49]. However, the orientation and especially the function of these muscles are the same. Muscle IItpm9 (IIItpm9) was described as two muscles lying very close to each other [9, 1, 4]; this could not be confirmed. Muscle IItpm2 (IIItpm2), which is present in adult Zygoptera [4], could not be confirmed. It might be a unique muscle for Zygoptera [cf. 9, 1].

vlm – Ventral longitudinal muscles (Figure 1)

Muscle IIvlm7 is one of the most characteristic muscles. It originates in the mesothorax, runs through the metathorax and inserts in the abdomen. Due to this characteristic course and the

identical attachment points, the homologization is straightforward [cf. 49]. The homologies of IIIvlm2 and IIIvlm3 are equally unequivocal; both have no homolog in the mesothorax. Asahina's [1] description of IIvlm6 is unclear; however, the figures of Maloeuf [9] and Asahina [1] are conclusive. Muscle IIvlm6 belongs to the mesothorax not to the metathorax as it had been described [cf. 9, 1]. Muscle Ivlm7 was described as M41 by Asahina [1] and as M42 by Maloeuf [9] and IIvlm7 is M42 in Asahina [1] and M41 in Maloeuf [9].

Conclusions

The homologization of the thorax musculature of Odonata with the generalized neopteran thorax and the established nomenclature of Friedrich & Beutel [49] was surprisingly straightforward [4]. However, the homology hypotheses that we present directly follow from our investigations. There might be different possibilities for interpreting some of the results; nevertheless, what we present seemed to be the most probable explanation, based on the currently available information.

The simplicity of our hypothesis is distinctly positive. In accordance with the "parsimony principle" or "Ockham's razor" [e.g. 57-59], we try to find the hypothesis that requires the smallest amount of assumptions to explain the observations. Since we assume that Pterygota are monophyletic (which is supported by numerous recent and not-so-recent phylogenetic analyses), we also have to assume that there once existed a last common ancestor of Pterygota that also represents its morphological ground pattern. From this ground-pattern the evolution of all pterygote subgroups started and therefore, there also should be a pattern of homologies between theses subgroups. These homologies have a good probability of being comparatively simple.

Starting from a ground pattern of Dicondylia (apterygote insect), a high numbered muscle setup is most likely. Zygentoma and even Archaeognatha show an almost uncountable number of single stranded muscles in their thorax [e.g. 60-62]. This assumption is supported by our findings; we found six muscles, which are not known for Neoptera. It seems to be quite probable that these muscles (Ipcm9, Itpm7 – Itpm11) belong to the pterygote ground pattern. Since the lifestyle of the wingless ancestor most likely resembled the apterygote (like Zygentoma) lifestyle, an ancestral occurrence is likely. For example muscle Ipcm9 has homologous muscles in the neopteran pterothorax (IIpcm5 and IIIpcm5; cf. Figure 3 and Friedrich&Beutel 2008]. This seems to be an interesting evolutionary trade-off, because IIpcm5 and IIIpcm5 are not present in Odonata. The pterygote ground pattern might show Ipcm9, IIpcm5 and IIIpcm5 as homologous muscles in pro-, meso- and metathorax. During

the evolution of Odonata IIpcm5 and IIIpcm5 were reduced and, on the other hand, Ipcm9 was reduced during the evolution of the Neoptera.

There is no extensive discussion of phylogenetic aspects for explaining the evolutionary circumstances and resulting relationships; since our work explicitly focuses on the question of the homology and evolution of the thoracic muscles of pterygota. Solving the problems at this level is essential for using muscle characters for phylogenetic analyses, which will be the next step that might be done based on the results and interpretations that are presented herein.

Methods

This study contains late instars of the Anisoptera species *Sympetrum vulgatum* (Linnaeus, 1758) (Libellulidae), *Aeshna affinis* Van Der Linden, 1820 (Aeshnidae) and *Cordulegaster bidentatus* Sélys, 1843 (Cordulegasteridae). From the collection of the Johann-Friedrich-Blumenbach-Institute of Zoology & Anthropology of the Georg-August-University in Göttingen, Germany. All regulations concerning the protection of free-living species have been followed.

The specimens were fixed in an alcoholic Bouin solution (= Duboscq-Brasil) [63]. After this initial fixation specimens were stored in 70% ethanol.

For the investigation of *Sympetrum vulgatum* synchrotron radiation micro computed tomography (SRμCT) was applied in order to generate data for the three-dimensional reconstruction of the structures of interest. Function and construction of a synchrotron were elaborately described by Betz et al. [64]. The data were generated at the Deutsches Elektronen Synchrotron (DESY) in Hamburg (Germany), using the beamline Petra III, (Proposal no. I-20090102, Aug. 2009, SB) and at the Swiss Light Source (SLS) in Villingen (Switzerland), using the beamline Tomcat, (Proposals no. 20080794, Mai 2009 and no. 20100088, Nov. 2010, TH).
Processing and visualization of the three-dimensional data were done with VGS Amira® 5.2. (Visage Imaging, Richmond, Australia).

For freehand preparation the specimens were halved along the body axis with a razorblade. The right side of the body was pasted into Paraplast, to preserve the shapes of the body during further preparations. Subsequently, the gut and all of the other tissues except the

musculature were removed. To study the specimens a reflected-light microscope (Stemi SV11 from Zeiss) was used with a camera lucida to assist drawing.

All figures were subsequently processed in Photoshop CS3, version 10.0.1 (Adobe System Inc., San José, USA).

In the present study the attachment points of the muscles are named such that the non-functional or non-moving end is called the point of origin and the functional or moving end is the point of insertion. The muscle names are formed accordingly. In a few cases this leads to differences in the muscle descriptions in comparison to other authors [e.g. 9, 1, 49] even though the same muscle is addressed.

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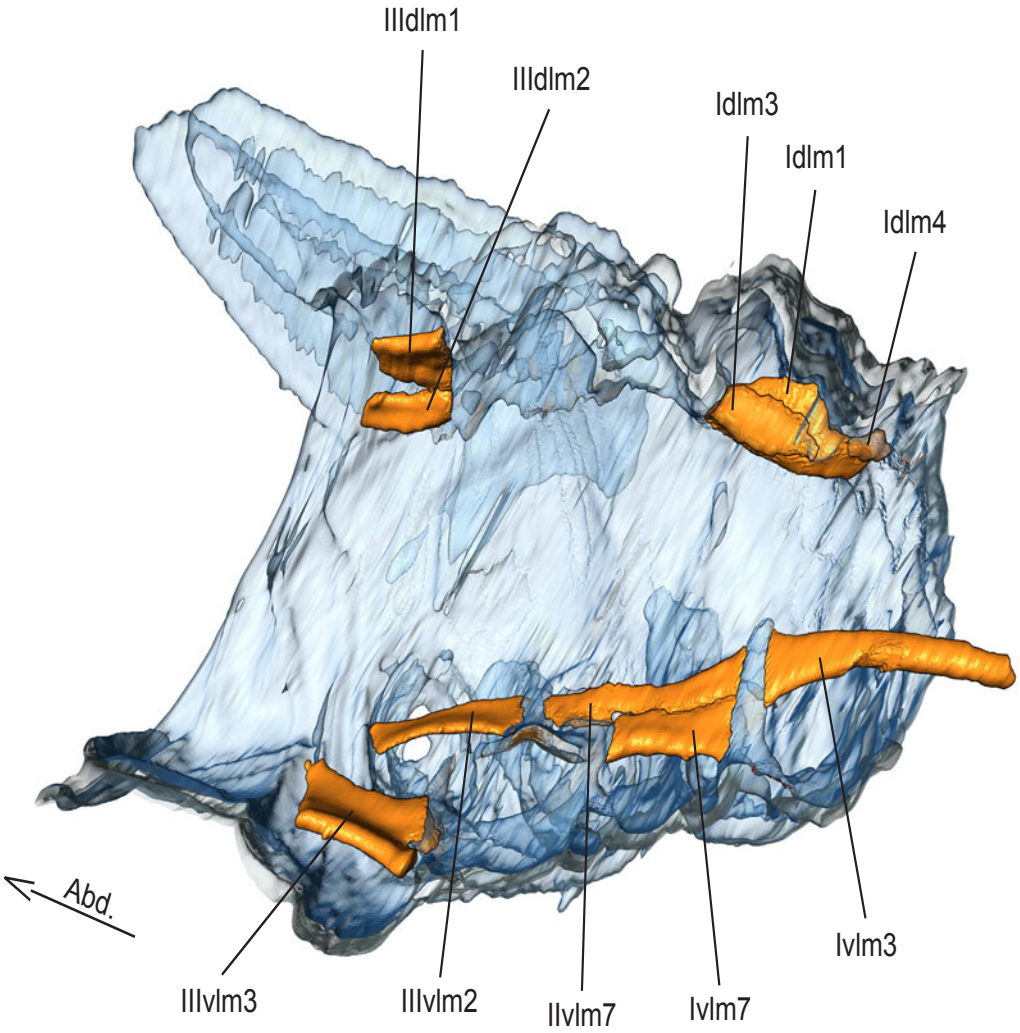

Figure 1: Dorsal longitudinal and ventral longitudinal musculature of *Sympetrum vulgatum*. 3D - reconstruction from SRμCT showing the left half of the thorax.
Abd - Abdomen, dlm - dorsal longitudinal muscle, vlm - ventral longitudinal muscle.

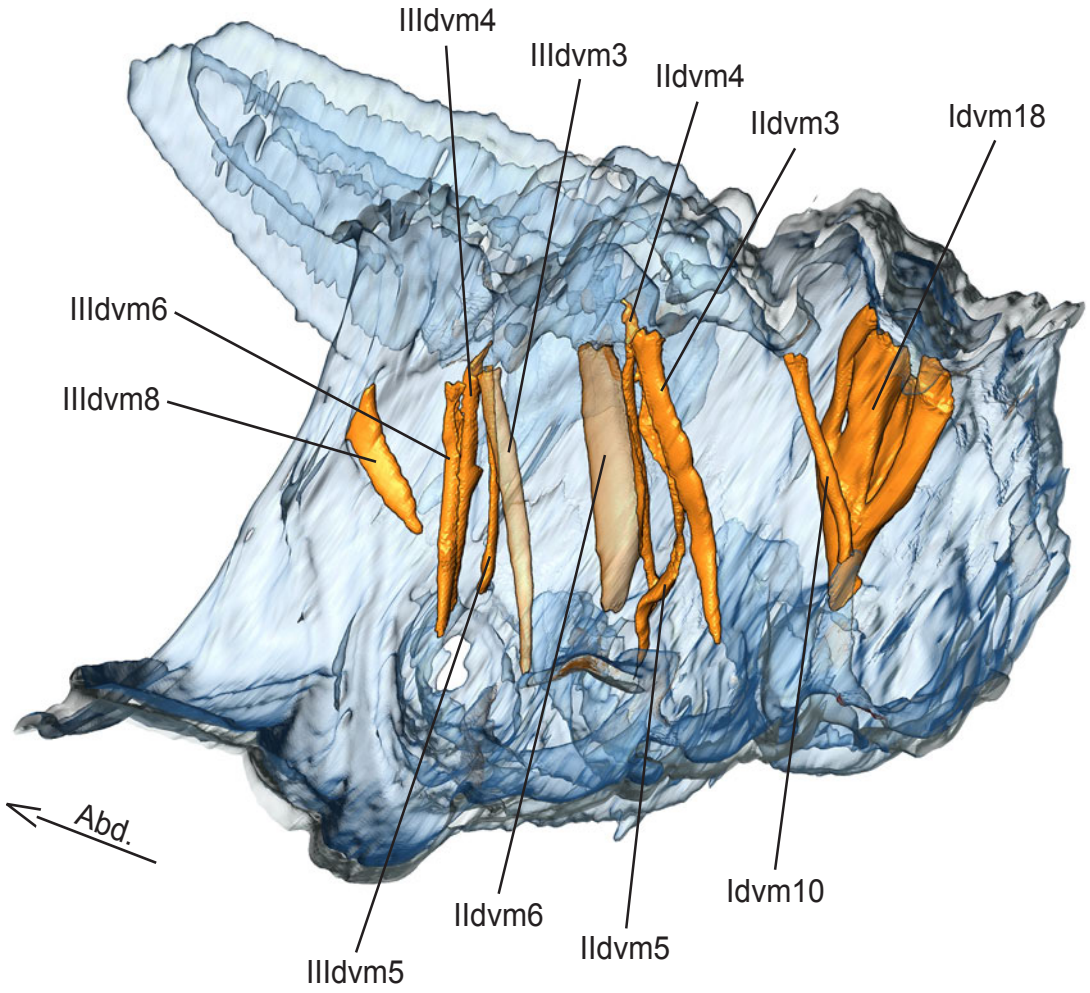

Figure 2: Dorso-ventral musculature of *Sympetrum vulgatum*.
3D - reconstruction from SRμCT showing the left half of the thorax.
Abd - Abdomen, dvm - dorso-ventral muscle.

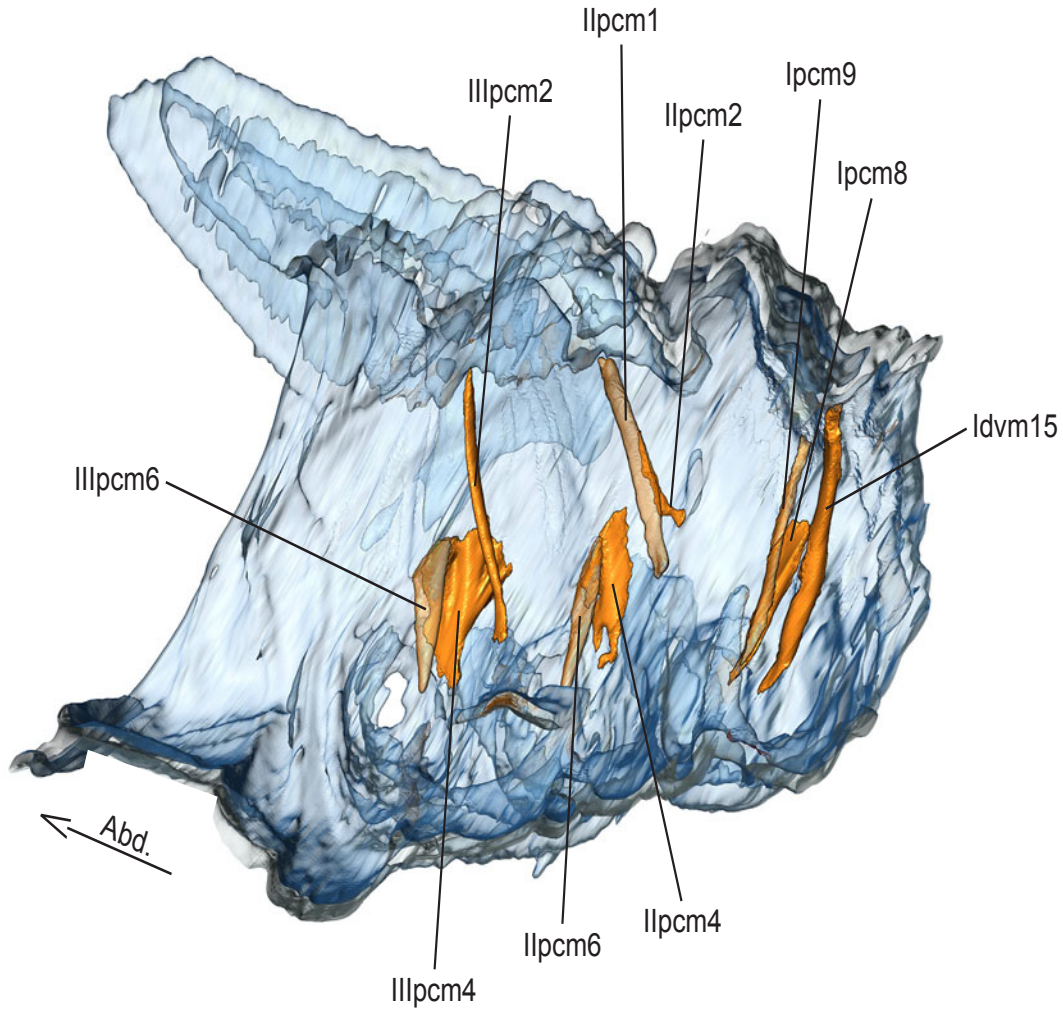

Figure 3: Pleuro-coxal musculature of *Sympetrum vulgatum*.
3D - reconstruction from SRμCT showing the left half of the thorax.
Abd - Abdomen, pcm - pleuro-coxal muscle.

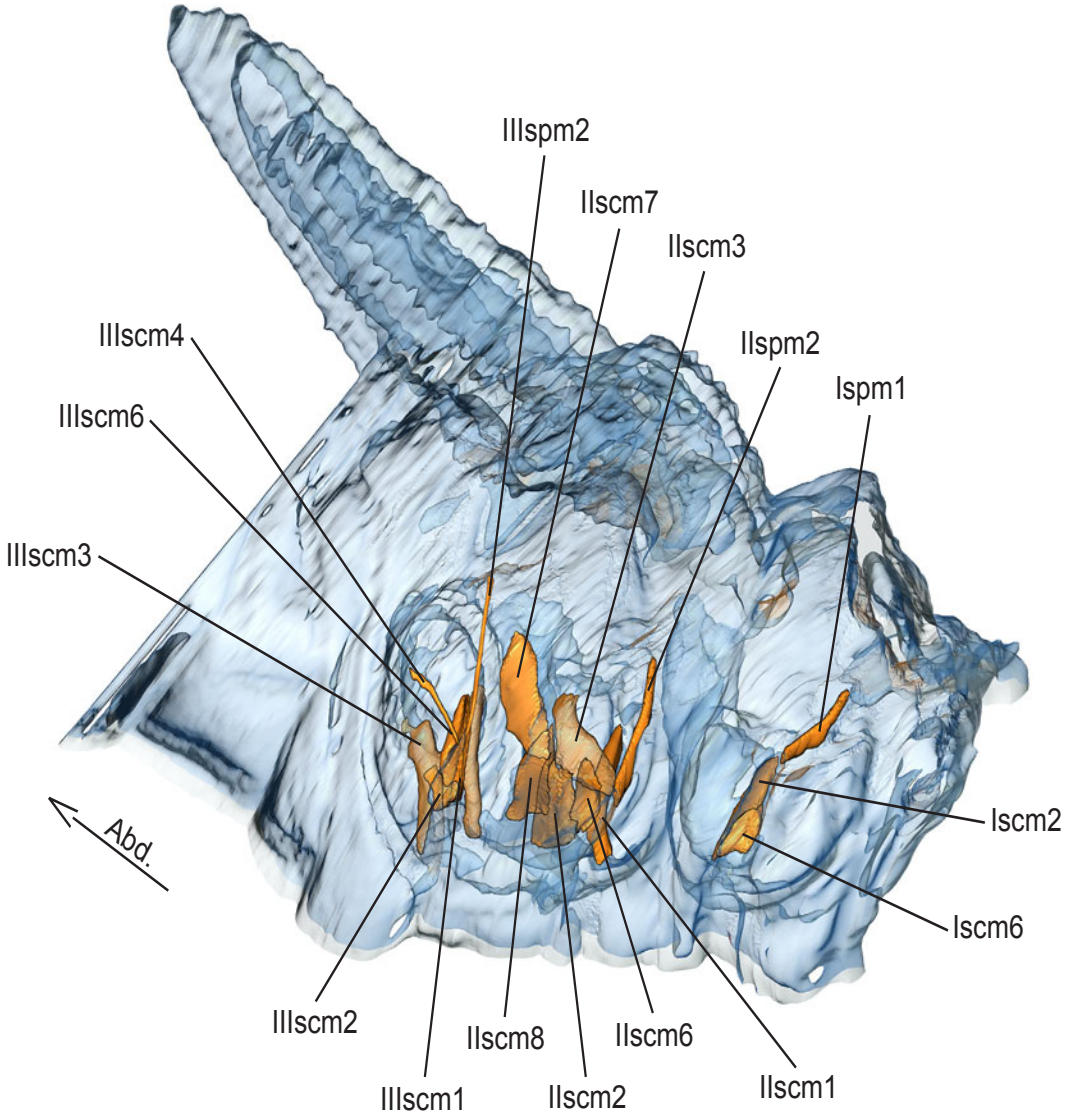

Figure 4: Sterno-coxal and sterno-pleural musculature of *Sympetrum vulgatum*.
3D - reconstruction from SRμCT showing the left half of the thorax.
Abd - Abdomen, scm - sterno-coxal muscle, spm - sterno-pleural muscle.

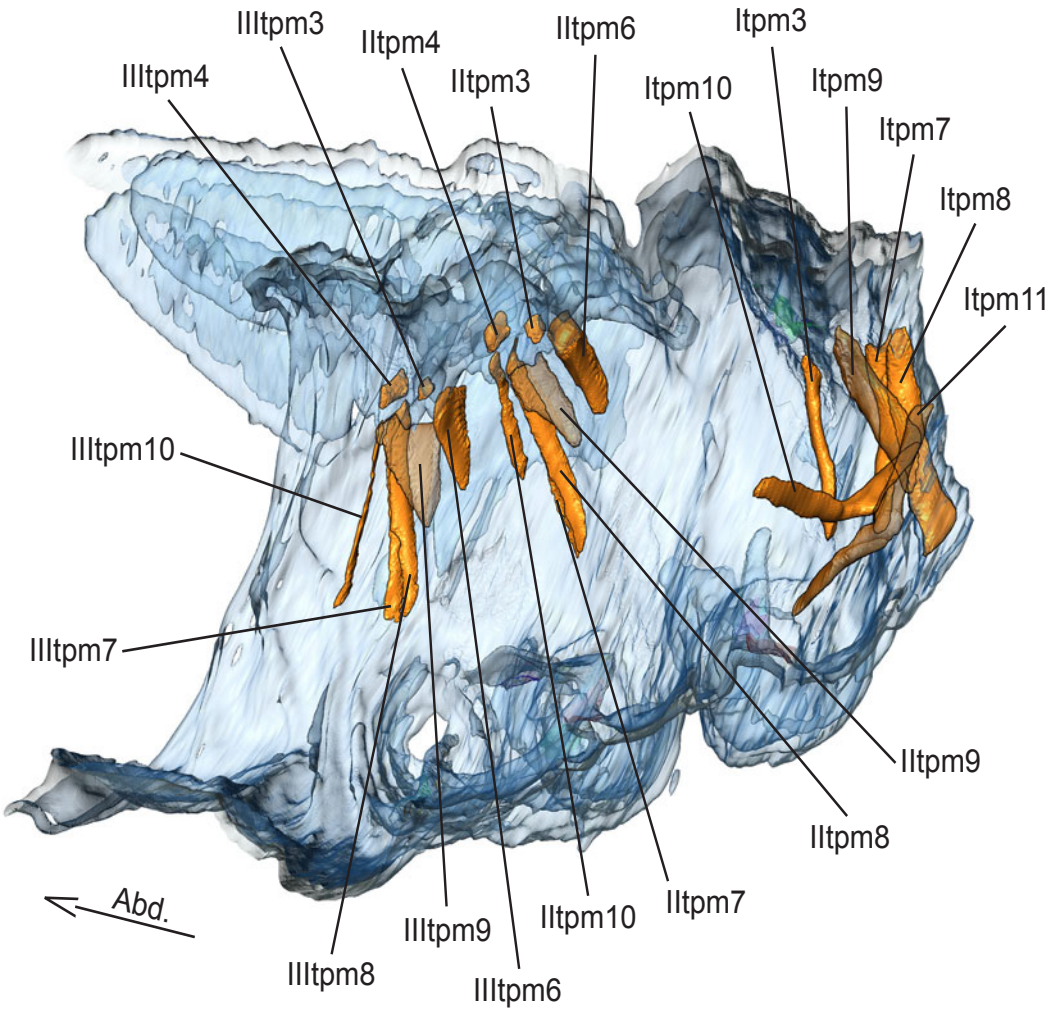

Figure 5: Tergo-pleural musculature of *Sympetrum vulgatum*.
3D - reconstruction from SRμCT showing the left half of the thorax.
Abd - Abdomen, tpm - tergo-pleural muscle.

Adittional file 1: Attachmentpoints of the thorax musculature of *Sympetrum vulgatum*

Abbrevation	Name	Origin	Insertion
Prothorax			
Dorsal longitudinal muscles			
Idlm1	Musculus prophragma-occipitalis	Apex of tergal apophysis 2	Median at the postocciput
Idlm3	M. prophragma-cervicalis	Tergal apophysis 1	Base of tergal apophysis 2
Idlm4	M. cervico-occipitalis dorsalis	Tergal apophysis 1	Median at postocciput
Dorsoventral muscles			
Idvm10	M. profurca-phragmalis	Apex of profurca	Apex of tergal apophysis 2
Idvm15	M. propleuro-coxalis superior	Anterolateral portion of tergite 1	Anterior procoxal rim
Idvm18	M. pronto-coxalis lateralis	Postero-lateral portion of tergite 1	Procoxal disk
Pleuro-coxal muscles			
Ipcm8	M. propleuro-trochanteralis	Episternum 1	Tendon of protrochanter
Ipcm9	M. protergro-trochanteralis	Lateral portion of tergite 1, close to the pleura.	Tendon of protrochanter
Sterno-coxal muscles			
Iscm2	M. profurca-coxalis posterior	External side of the base of profurca	Posterior procoxal rim
Iscm6	M. profurca-trochanteralis	External side of the base of profurca	Tendon of protrochanter
Sterno-pleural muscles			
Ispm1	M. profurca-apodemalis	Apex of profurca	Apodem of propleura
Tergo-pleural muscles			

IIdvm6	M. mesocoxa-subalaris	Lateral part of tergite 2	Postero-lateral apodem of mesocoxa
Pleuro-coxal muscles			
IIpcm1	M. mesanepisterno-trochantinalis	Preepisternum 2	Lateral of the tergal apophysis 2 at tergite 2
IIpcm2	M. mesobasalare-trochantinalis	Base of preepisternal apodem 2	Lateral of the tergal apophysis 2 at tergite 2
IIpcm4	M. mesanepisterno-coxalis posterior	Interpleuralridge 2	Antero-external part of mesocoxa
IIpcm6	M. mesopleura-trochanteralis	Dorsal part of Katepisternum 2	Tendon of mesotrochanter
Sterno-coxal muscles			
IIscm1	M. mesofurca-coxalis anterior	Lateral base of Mesofurca	Antero-external ridge of mesocoxa
IIscm2	M. mesofurca-coxalis posterior	Lowermost part of mesofurca	Postero-lateral apodem of mesocoxa
IIscm3	M. mesofurca-coxalis medialis	Lateral base of mesofurca	Postero-lateral apodem of mesocoxa
IIscm6	M. mesofurca-trochanteralis	Latero-external side of mesofurca	Tendon of mesotrochanter
IIscm7	M. mesospina-metacoxalis	Preepisternal apodem	Anterolateral edge of metacoxa
Sterno-pleural muscles			
IIspm2	M. mesofurca-pleuralis	Apex of mesofurca	Interpleural ridge 2
Tergo-pleural muscles			
IItpm3	M. mesonoto-basalaris	Dorsal side of mesowing bud, anterior to the origin of IItpm4	Ventral side of mesowing bud, anterior to origin of IItpm4
IItpm4	M. mesonoto-pleuralis anterior	Dorsal side of mesowing bud, posterior to the origin of IItpm3	Ventral side of mesowing bud, posterior to the origin of IItpm3

IItpm6	M. mesonoto-pleuralis posterior	Upper portion of interpleural ridge 2	Antero-dorsal edge of mesowing bud
IItpm7	M. mesanepisterno-axillaris	Ventral part of epimeron 2	Lateral edge of mesowing bud
IItpm8	M. mesepimero-axillaris secundus	Ventral part of epimeron 2	Lateral edge of mesowing bud
IItpm9	M. mesepimero-axillaris tertius	Dorsal part of epimeron 2	Inner side of ventral portion of mesowing bud
IItpm10	M. mesepimero-subalaris	Interpleural ridge 2	Lateral edge of mesowing bud
Ventral longitudinal muscles			
IIvlm6	M. mesospina-abdominosternalis	Posterior part of preepisternalapodem 3	Antecostal apodem
IIvlm7	M. mesofurca-abdominosternalis	Mesofurca	Within the Abdomen
Metathorax			
Dorsal longitudinal muscles			
IIIdlm1	M. mesophragma-metaphragmalis	Tergal apophys 4	Transversal ridge between abdomen and thorax
IIIdlm2	M. metanoto-phragmalis	Tergal apophysis 4	Transversal ridge between abdomen and thorax
Dorsoventral muscles			
IIIdvm1	M. mesonoto-sternalis	Base of Metafurca	Postero-lateral edge of metawing bud
IIIdvm3	M. metanoto-trochantinalis	Base of Metafurca	Antero-lateral edge of metawing bud
IIIdvm4	M. metanoto-coxalis anterior	Anterio-lateral edge of metaocoxa	Antero-lateral edge of metawing bud
IIIdvm5	M. metanoto-coxalis posterior	Metacoxaldisk	Antero-lateral edge of metawing bud

IIdvm6	M. metacoxa-subalaris	Lateral part of tergite 3	Postero-lateral part of metacoxa
IIIdvm8	M. metanoto-phragmalis	Dorsal portion of the posterior ridge of epimeron 3	Posterior end of metafurca
Pleuro-coxal muscles			
IIIpcm1	M. metanepisterno-trochantinalis	Preepisternum 3	Lateral of the tergal apophysis 3 at tergite 3
IIIpcm2	M. metabasalare-trochantinalis	Base of Preepisternal apodem 3	Lateral of the tergal apophysis 3 at tergite 3
IIIpcm4	M. metanepisterno-coxalis posterior	Interpleuralridge 3	Antero-external part of metacoxa
IIIpcm6	M. mesopleura-trochanteralis	Dorsal part of Katepisternum 3	Tendon of metatrochanter
Sterno-coxal muscles			
IIIscm1	M. metafurca-coxalis anterior	Lateral base of Metafurca	Antero-external ridge of metacoxa
IIIscm2	M. metafurca-coxalis posterior	Lowermost part of metafurca	Postero-lateral apodem of metacoxa
IIIscm3	M. metafurca-coxalis medialis	Lateral base of metafurca	Postero-lateral apodem of metacoxa
IIIscm4	M. metafurca-coxalis lateralis	Apex of metafurca	Lateral base of metacoxa, at the border of pleurite
IIIscm6	M. metafurca-trochanteralis	Latero-external side of metafurca	Tendon of metatrochanter
Sterno-pleural muscles			
IIIspm2	M. metafurca-pleuralis	Apex of metafurca	Interpleural ridge 3
Tergo-pleural muscles			
IIItpm3	M. metanoto-basalaris	Dorsal side of metawing bud, anterior to the origin of IIItpm4	Ventral side of metawing bud, anterior to origin of IIItpm4

IIItpm4	M. metanoto-pleuralis anterior	side of metawing bud, posterior to the origin of IIItpm3	Ventral side of metawing bud, posterior to the origin of IIItpm3
IIItpm6	M. metanoto-pleuralis posterior	Upper portion of interpleural ridge 3	Antero-dorsal edgel of metawing bud
IIItpm7	M. metanepisterno-axillaris	Ventral part of epimeron 3	Lateral edge of metawing bud
IIItpm8	M. metapimero-axillaris secundus	Ventral part of epimeron 3	Lateral edge of metawing bud
IIItpm9	M. metapimero-axillaris tertius	Dorsal part of epimeron 3	Inner side of ventral portion of metawing bud
IIItpm10	M. metapimero-subalaris	Interpleural ridge 3	Lateral edge of metawing bud
Ventral longitudinal muscles			
IIIvlm2	M. mesofurca-abdominosternalis	part of Metafurca (close to the prefurca invagination)	Within the abdomen (second abdominal sternite)
IIIvlm3	M. metaspina-abdominosternalis	Poststernum 3	the abdomen (second abdominal sternite)

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

Friedrich & Beutel (2008)	this study	Büsse et al. (2013)	Asahina (1954)	Willkommen (2008)	Wittig (1955)	Matsuda (1970)
Prothorax						
Idlm1	x	?	3	?	I dlm 10	op-t 3
Idlm2	-	?	-	?	0 dlm 1	op-t 2
Idlm3	x	?	2	?	I dlm 11b	cv(d)-t 1, t 14
Idlm4	x	?	1	?	0 dlm 2	op-t 1
Idlm5	-	?	-	?	I dlm 12	t 12
Idlm6	-	?	-	?	I dlm 12?	t 13
Idvm1	-	?	-	?	0 lm 7	op-cv 1
Idvm2	-	?	-	?	0 lm 7	op-cv 2
Idvm3	-	?	-	?	0 lm 8	op-cv 3
Idvm4	-	?	-	?	0 lm 5	t-s(cv) 1?
Idvm5	-	?	-	?	-	t-cv 1
Idvm6	-	?	-	?	0 lm 6	t-cv 2
Idvm7	-	?	-	?	-	t-cv 3
Idvm8	-	?	-	?	-	t-s(cv) 9
Idvm9	-	?	-	?	-	op-s 2, p-s 3
Idvm10	x	?	-	?	I ism 22	t-s 1
Idvm11	-	?	-	?	I ism 24	t-s 8
Idvm12	-	?	-	?	-	t-s 2
Idvm13	-	?	-	?	I dvm 15	t-ti(cx) 2
Idvm14	-	?	-	?	I dvm 16	t-ti(cx) 3
Idvm15	x	?	13	?	I dvm 17?	t-ti(cx) 1
Idvm16	-	?	-	?	I dvm 19	t-cx 5
Idvm17	-	?	-	?	I dvm 20	t-cx 6, t-cx 7
Idvm18	x	?	14 & 15	?	I dvm 21	t-cx 8
Idvm19	-	?	-	?	I dvm 18	t-tr 1

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

ltpm1	-	?	-	?	-	op-p 2
ltpm2	-	?	-	?	0 lm 9	op-p 1, t-p 3
ltpm3	x	?	12	?	l tpm 25	-
ltpm4	-	?	-	?	l tpm 26	t-p 14?
ltpm5	-	?	-	?	l tpm 27?	t-p 15?
ltpm6	-	?	-	?	-	t-p 1, t-p 2
-	ltpm7	?	4	?	?	?
-	ltpm8	?	5	?	?	?
-	ltpm9	?	7	?	?	?
-	ltpm10	?	9	?	?	?
-	ltpm11	?	10	?	?	?
lspm1	x	?	-	?	l zm 34	p-s1
lspm2	-	?	-	?	-	p-s 2
lspm3	-	?	-	?	-	p-s 6
lspm4	-	?	-	?	-	p-s 7
lspm5	-	?	-	?	-	p-s 5
lspm6	-	?	-	?	-	p-s 4
lspm7	-	?	-	?	-	p-s 10
lpcm1	-	?	-	?	-	cv-cx 3
lpcm2	-	?	-	?	-	cv-cx 1, cv-cx 2
lpcm3	-	?	-	?	-	p-ti(cx) 1
lpcm4	-	?	-	?	-	p-cx 4
lpcm5	-	?	-	?	l cpm 28	p-cx 5
lpcm6	-	?	-	?	-	p-cx 6, p-cx 9
lpcm7	-	?	-	?	-	p-cx 7
lpcm8	x	?	18	?	l cpm 29	p-tr 1, p-tr 2
-	lpcm9	?	17	?	?	?
lvlm1	-	?	-	?	0 vlm 4	cv-s 1, cv-s 4?
lvlm2	-	?	-	?	-	op-cv(v) 4

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

Ivlm3	x	?	11	?	0 vlm 3	s 1, s 2
Ivlm4	-	?	-	?	I vlm 14	s 14, s16
Ivlm5	-	?	-	?	-	s 17
Ivlm6	-	?	-	?	-	s 15
Ivlm7	x	?	41	?	I vlm 13	s 13
Ivlm8	-	?	-	?	-	s 11
Ivlm9	-	?	-	?	-	s 12
Iscm1	-	?	-	?	I bm 30	s-cx 5
Iscm2	x	?	16	?	I bm 33	s-cx 3
Iscm3	-	?	-	?	I bm 32	s-cx 6
Iscm4	-	?	-	?	-	s-cx 2
Iscm5	-	?	-	?	-	s-cx 4
Iscm6	x	?	19	?	I bm 31	s-tr 1
Iscm7	-	?	-	?	-	s-cx 1, s-cx 7
Mesothorax						
IIdlm1	x	x	25	MT.m	II dlm 35	t 14
IIdlm2	-	-	-	S.LP?m	II dlm 36, II dlm 37	t12, t13
IIdlm3	-	-	-	S.Esm	-	t-p 5, t-p 6
IIdvm1	x	x	23'	S.CmA	II dvm 40	t-ti 1, t-ti 2
IIdvm2	-	-	-	S.CmA	II dvm 41	t-ti 3
IIdvm3	x	x	23	-	-	t-cx 5
IIdvm4	x	x	26	PSL.Cm, S.CmP	II dvm 43	t-cx 6, t-cx 7
IIdvm5	x	x	27	SA.Cm, SA.Fm	II dvm 43	t-cx 8
IIdvm6	x	-	-	S.Trm	II cpm 53	t-tr 1
IIdvm7	-	-	-	-	II dvm 42	t-s 1
IIdvm8	-	-	-	-	II ism 44	t-s 8, t-s 7 ?
IIdvm9	-	-	-	A?.Pm	-	t-p 3
IItpm1	-	-	-	BA.Pm	II tpm 46a	t-p 4, t-p 20

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIltpm2	-	x	-	-	II tpm 47	t-p 7, t-p 8, t-p 9
IIltpm3	x	-	-	SrA.Pm, Ax.Pml	II tpm 46b ?	t-p 10, t-p 11, t-p 18
IIltpm4	x	x	28	-	-	t-p 12
IIltpm5	-	-	-	-	-	t-p 15
IIltpm6	x	x	31	-	II tpm 49	t-p 13
IIltpm7	x	x	33	-	II tpm 48	-
IIltpm8	x	x	32	Ax.PmS	-	t-p 14
IIltpm9	x	x	29/30	-	-	t-p 16
IIltpm10	x	x	34	-	II ppm 56	t-p 19
IIltpm11	-	-	-	-	-	t-p 17
IIltpm12	-	-	-	-	-	p 1
IIltpm13	-	-	-	-	?	?
IIppm1	-	-	-	-	II im 65a	p 2
IIppm2	-	-	-	-	II pm 54a, b	p 3
IIspm1	-	-	-	-	II ppm 55	p-s 1
IIspm2	x	-	35	-	II zm 61a	p-s 2
IIspm3	-	-	-	-	-	p-s 6
IIspm4	-	-	-	-	-	p-s 7
IIspm5	-	-	-	-	-	p-s 3
IIspm6	-	-	-	-	-	p-s 9
IIspm7	-	-	-	-	-	p-s 5
IIspm8	-	-	-	-	-	p-ti(cx) 1
IIpcm1	x	x	21	-	-	p-ti(cx) 2, p-ti(cx) 3
IIpcm2	x	x	22	-	II cpm 51	p-cx 4, p-cx 6
IIpcm3	-	-	-	P.Cm	-	p-cx 5
IIpcm4	x	x	36	BA.Trm	II cpm 52	p-tr 2
IIpcm5	-	-	-	P.Trm	II cpm 50	p-tr 1
IIpcm6	x	x	39	Fm	-	s14, s16
IIvlm1	-	-	-	-	-	s 15

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIvlm2	-	-	-	iFm	-	s 13
IIvlm3	-	-	-	-	II vlm 38	s 11
IIvlm4	-	-	-	-	-	s 12
IIvlm5	-	-	-	-	II vlm 39	p-s 13
IIvlm6	x	-	68	-	-	p-s 10
IIvlm7	x	x	41	F.CmA	-	s-cx 5
IIscm1	x	-	-	-	II bm 57	s-cx 3
IIscm2	x	-	-	-	II bm 60	s-cx 6
IIscm3	x	x	38	F.CmP	II bm 59	s-cx 2
IIscm4	-	-	-	-	II zm 61b	s-cx 4
IIscm5	-	-	-	-	-	s-tr1
IIscm6	x	x	40	-	II bm 58	s-cx 1
IIscm7	x	-	-	-	-	s-cx 7
Metathorax						
IIIdlm1	x	x	45	MT.m	III dlm 35	t 14
IIIdlm2	x	x	45'	S.LP?m	III dlm 36, III dlm 37	t 12, t13
IIIdlm3	-	-	-	-	III dvm 40	?
IIIdvm1	-	x	46'	S.Esm	III dvm 41	t-p 5, t-p 6
IIIdvm2	-	-	-	S.CmA	-	t-ti 1, t-ti 2
IIIdvm3	x	x	46	S.CmA	III dvm 43	t-ti 3
IIIdvm4	x	x	48	-	III dvm 43	t-cx 5
IIIdvm5	x	x	49	PSL.Cm, S.CmP	III cpm 53	t-cx 6, t-cx 7
IIIdvm6	x	-	46	SA.Cm, SA.Fm	III dvm 42	t-cx 8
IIIdvm7	-	-	-	-	III ism 44	t-tr 1
IIIdvm8	x	x	67	-	III tpm 46a	t-s 1
IIItpm1	-	-	-	A?.Pm	III tpm 47	t-p 3
IIItpm2	-	x	-	BA.Pm	III tpm 46b	t-p 4, t-p 20
IIItpm3	x	-	-	-	-	t-p 7, t-p 8

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIItpm4	x	x	50	SrA.Pm, Ax.Pml	-	t-p 10, t-p 11, t-p 18
IIItpm5	-	-	-	-	III tpm 49	t-p 12
IIItpm6	x	x	53	-	III tpm 48	t-p 15
IIItpm7	x	x	55	-	-	t-p 13
IIItpm8	x	x	54	-	-	-
IIItpm9	x	x	51/52	Ax.PmS	III ppm 56	t-p 14
IIItpm10	x	x	56	-	-	t-p 16
IIItpm11	-	-	-	-	-	t-p 19
IIItpm12	-	-	-	-	-	t-p 17
IIItpm13	-	-	-	-	?	?
IIIppm1	-	-	-	-	III im 65a	p 1
IIIppm2	-	-	-	-	III ppm 54a, b	p 2
IIIspm1	-	-	-	-	III ppm 55	p 3
IIIspm2	x	-	57	-	III zm 61	p-s 1
IIIspm3	-	-	-	-	-	p-s 7
IIIspm4	-	-	-	-	-	p-s 9
IIIspm5	-	-	-	-		p-s 5
IIIspm6	-	-	-	-	?	?
IIIpcm1	x	x	43	-	-	p-ti(cx) 1
IIIpcm2	x	x	44	-	III cpm 51	p-ti(cx) 2, p-ti(cx) 3
IIIpcm3	-	-	-	-	-	p-cx 4, p-cx 6
IIIpcm4	x	x	58	P.Cm	III cpm 52	p-cx 5
IIIpcm5	-	-	-	BA.Trm	III cpm 50	p-tr 2
IIIpcm6	x	x	62	P.Trm	-	p-tr 1
IIIpcm7	-	-	-	-	-	p-cx 8
IIIvlm1	-	-	-	Fm	-	s 14, s16
IIIvlm2	x	x	65	-	III vlm 64	s 20
IIIvlm3	x	x	66	-	-	s 12
IIIscm1	x	-	-	F.CmA	III bm 57	s-cx 5

Additional file 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIIscm2	x	-	-	-	III bm 60	s-cx 3
IIIscm3	x	x	61	-	III bm 59	s-cx 6
IIIscm4	-	-	-	F.CmP	-	s-cx 2
IIIscm5	-	-	-	-	-	s-cx 4
IIIscm6	x	x	63	-	III cpm 58	s-tr 1

7. A Taxonomic Review of *Epiophlebia laidlawi* (Insecta: Odonata) – Including Remarks on Phylogeny

7.1. Contribution to this Manuscript

Conceived and designed the experiments: SB.
Performed the experiments: SB.
Analyzed the data: SB.
Contributed reagents/materials/analysis tools: SB.
Wrote the paper: SB.

7.2. Manuscript

Büsse S (in preparation) A Taxonomic Review of *Epiophlebia laidlawi* (Insecta: Odonata) – Including Remarks on Phylogeny.

A Taxonomic Review of *Epiophlebia laidlawi* (Insecta: Odonata) – Including Remarks on Phylogeny

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Keywords: Epiophlebidae; "Anisozygoptera"; Species Status;
Adult of *Epiolphlebia laidlawi*.

Introduction

The Odonata represent one of the oldest groups of winged insects (e.g. Erasipteron larischi Pruvost, 1933 from the Upper Carboniferous). Generally, Odonata can be grouped into three subordinated taxa: Two well known groups, dragonflies (Anisoptera) and damselflies (Zygoptera), as well as the not commonly known and enigmatic group *Epiophlebia* (Asahina 1954, Hennig 1959, Büsse et al. 2012). Historically, the extant species of *Epiophlebia* were grouped together with fossil taxa, forming the "Anisozygoptera" (e.g. Nell 1993). This name relates to the conspicuous mixture of anisopteran and zygopteran characters found in its extant species (Asahina 1954, Rüppell & Hilfert 1993, Xylander & Günther 2003, Büsse et al. 2012). This term is however avoided herein, because Nel (1993) already showed that the "Anisozygoptera" are a paraphyletic assemblage.

The species of *Epiophlebia* seem to represent the most ancestral character distribution of all known Odonata (Blanke et al. 2013). *Epiophlebia* is neither part of the damselflies nor the dragonflies (e.g. Bybee 2008), but is the sister group of the Anisoptera forming the monophylum Epiprocta (Carle 1982, Bechly 1996, Lohmann 1996, Trueman 1996, Misof et al. 2001, Rehn 2003, Bybee et al. 2008, Gade et al. 2011). Still more, characters of recent *Epiophlebia* are present in Zygoptera and the stem-group of Epiprocta, but neither in the stem-group of Anisoptera and nor in the crown-group of Anisoptera. Phylogenetically, it is one of the closest recent representatives, positioned at the split of Anisoptera and Zygoptera of all know Odonata, including fossils (e.g. Lohmann 1996).The well-known species are *Epiophlebia superstes* (Sélys, 1889) and *Epiophlebia laidlawi* Tillyard, 1921 in contrast to the recently described species *Epiophlebia sinensis* Li and Nel, 2011 and *Epiophlebia diana* Carle, 2012. In the latter description a new sub-

genus was erected - *Rheoepiophlebia* (Carle 2012), but has gone unreferred to in this publication.

The heyday of the ancestors of *Epiophlebia* was in the Mesozoic (Nel et al. 1993; Carpenter 1993). Recently, the extant species of *Epiophlebia* are considered relict species (Asahina 1954, Davies 1992, Mahato 1993). Their disjunct distribution (Büsse et al. 2012) is restricted to large areas of Japan in case of *E. superstes* (Asahina 1954, Tabaru 1984); to the Himalayas in *E. laidlawi* (Asahina 1961, 1963, Brockhaus & Hartmann 2009, Nesemann et al. 2011) and to China in *E. sinensis* (Li et al. 2011) as well as in *E. diana* (Carle 2012).

While the Japanese *E. superstes* is well studied and was thoroughly described by Asahina (1954) and the head additionally by Blanke et al. (2013) Little is known about *E. laidlawi*, which was described by Tillyard in 1921 based on a single nymph. The adult of *E. laidlawi* was studied by Asahina (1963) and Davies (1992) and the nymphs by Asahina (1961). All these descriptions of *E. laidlawi* are rather superficial.
Asahina (1963: p. 18) mentioned the following characters of adult *E. laidlawi* to be different from *E. superstes*: "Colouration more brownish with fewer yellow markings; male genitalia differ especially in the shape of the hamulus posterioris; apical process of the eighth sternite less developed in the female; wings slightly longer".
Asahina (1961: p. 445) listed the following differences for *E. laidlawi* nymphs: "Body size slightly larger; third antennal segment relatively longer and thicker than in E. *superstes*; antero-lateral angle of the pronotum rounded in an obtuse angle in *E. laidlawi*, angle in *E. superstes* sharply pointed anteriorly. The fore femur is longer and much wider in *E. laidlawi* and the lateral spines of abdominal segments seven through nine are well protruded into a round process in *E. superstes* and undeveloped in *E. laidlawi*."
Four of these nymphal characters could not be confirmed by supplementary study of morphology in Büsse et al. (2012). The “morphological characters” from Nesemann et al. (2011) refer to nymphal colour patterns, about which no satisfactory taxonomic statement can be made.

The species status of the two recently described species are questionable (e.g. Büsse et al. 2012); the discrimination of *E. sinensis* based above all on a rufism, a reddish coloration, on abdominal segment 6 through 10 (Li et al. 2011). The discrimination of *Epiophlebia diana* is based on characters like “premental cleft longer than ventral width of palp at midlength” or “abdominal stridulatory file of segment 7 ca. ½ length of segment” (Carle 2012 p: 78) compared to *E. superstes*. Numerical characters by a sample size of two are questionable because the variation of characters within an odonatan population could be remarkable (e.g. Corbet 1999). Furthermore, the variation of characters within a nymphal stage of Odonata, caused for example by temperature or nutritional conditions, could be enormous (e.g. Corbet 1999). *Epiophlebia* are upper-

most in the odonatological mind since their first description by Sélys, 1889. The recent discoveries of *E. sinensis* and *E. diana* (Li et al 2011, Carle 2012) and the DNA analysis of three *Epiophlebia* species (Büsse et al. 2012) has provided new insights into one of the world's most enigmatic dragonfly groups.

The results of Büsse et al. (2012) show an unexpected similarity of DNA sequences (18s,28s, ITS1, ITS2, CO2) in the *Epiophlebia* species investigated and suggest that *E. superstes*, *E. laidlawi* and *E. sinensis* are solely an allopatric population of *E. superstes*. They also indicated that taxonomic revision and reconsideration of the species status is inevitable. The aim of this publication is therefore to elucidate the morphology of *Epiophlebia laidlawi*, of the immensely under-recorded adults in particular and compares morphological characters with the other three species, especially *E. superstes*. The species status and phylogeny of the *Epiophlebia* species will be discussed as well.

Material & Methods

The following adults and nymphs of *Epiophlebia laidlawi* Tillyard, 1921 were included:

- 11 nymphs, two adult females and three adult males from the Davies Collection of the University Museum of Zoology, University of Cambridge, UK.
- One adult male from the Collection of the Dragonfly Kingdom Nature Park in Shimanto City, Japan.
- 10 nymphs from the collection of the Hindu Kush Himalayan Benthological Society, Kathmandu, Nepal.

The following adults and nymphs of *Epiophlebia superstes* (Sélys, 1889) were included for comparison:

- 9 nymphs, one adult female and one adult male from the Collection of Systematic Entomology, Graduate School of Agriculture, Hokkaido University Sapporo, Japan.
- Two adult females and two adult males from the Davies Collection of the University Museum of Zoology, University of Cambridge, UK.

Closer information of the investigated specimens are available in supplementary table (Sup.1). All the regulations concerning the protection of free-living species were followed. Photographs were taken with a Keyence-Digitalmicroscope (VHX600) at the Department of Mineralogy at the Georg-August University of Goettingen or at the respective museum using a Canon 550D with a Metz MB 15 MS-1 Makroslave digital ring flash; entirely mounted on a Cullmann NANOMAX 200T traveler tripod to perform stacking photography using an Apple Macintosh Airbook and Helicon Remote and

Helicon Focus software (HeliconSoft Ltd., Kharkov, Ukraine). All images were subsequently processed in Photoshop CS3 (Adobe System Inc., San José, USA).

Results

Investigation of body parts that are difficult to access was only possible in part. Dissection of the rare specimens was unauthorized. In undissected condition, some data has therefore remained unavailable. The manuscript provides figures (Fig. 1-X) showing the major differences between *E. laidlawi* and *E. superstes* as well as some overview photographs in the supplement (Suppl. 1-X). It does not provide figures and descriptions for a comprehensive picture of the basic morphology of *Epiophlebia*. The copious work of Asahina (1954) is recommended for this. All characters are developed in the male and female specimens unless the contrary is mentioned.

Morphological Characters of Adult *Epiophlebia laidlawi*

Head characters (Figure 1 & 2)

The strongly sclerotized orthognathous head is obviously covered with a brownish coat of hair. The head is spherical in shape and mainly composed of laterally located, strongly convex compound eyes that are not medially connected. All ommatidia show a generally uniform size. The dorsal part of the occiput, between the compound eyes, steeply declines to the foramen. The occipital tubercles are distinctly developed, while seeming to be less developed in the female. The postfrontal and premandibular sutures seem to merge into each other; the junction is not distinctly separable. The premandibular suture runs around the posterior part of the compound eyes and dorsally anterior to the mandibular articulation. The most prominent character of the head of *Epiophlebia* is the strongly elevated fan-shaped transverse ridge (vertex) of the postfrons, which is v-shaped and medially nearly pointed from a dorsal view. From a frontal view, a small median bulge at the base of the vertex is visible. The antefrons is semicircular and medially vaulted, forming a medial tapered ridge. The clypeus is divided into anteclypeus and postclypeus. The latter is strongly sclerotized, whereas the anteclypeus is membranous. The sclerites of the anteclypeus are weakly developed. The clypeofrontal suture is planar but distinctly visible The shape of the labrum might be unique within the Odonata; it is shortish but broad, with the lateral sides protruding strongly transversally. The brownish hair is thin but covers the labrum completely. The vertex separates the normally triangular co-occuring ocelli. The median ocellus is located in front of this – atypical for Odonata – elevated vertex; the lateral ocelli vanish behind it. The antennae are for Odonata typically reduced and comprise five antennomers. The scape is cylindrical and arises from the postero-lateral part of the antefrons. The pedicle is broad, distinctly flattened dorso-ventrally and up to four times longer than the scape. Long hair covers the flattened pedicle at the lateral edges. The other

three antennomers – the flagellum – are developed and extremely slender. The first flagellum segment is approximately half as long as the pedicel, the second segment is a third or half as long as the first and the third segment is again half as long as the second. The mandibles are only vaguely identifiable; viewed dorsally they are triangular. The anterior mandibular articulation is located at the tri-border region of the postclypeus, the antefrons and the gena. The posterior mandibular articulation is located at the hindmost part of the mandible at the end of the premandibular suture. The basic parts of the maxillae are identifiable but hardly describable in detail. However, they show the same characteristics as in *E. superstes* (cf. Asahina 1954, Blanke et al. 2012). The labium covers the mouthparts dorsally and consists of the postmentum and prementum. The postmentum is enlarged. The small end hooks (Asahina 1954, Blanke et al. 2012) are covered, but the movable hooks, which conclude the labial palps, are prominent. The glossa and paraglossa are merged and form the median lobe (Blanke et al. 2012); the beginnings of both structures are recognizable between the movable hooks.

No yellow markings, only different shades of brown and black characterize the head of *E. laidlawi* (Supl. 2-4).

Thoracic Characters

Covered as they are by the eyes, the cervical sclerites are not visible. The prothoracic tergite is straight and sparsely developed. The anterior lobe is small and ridge-like and the pronotum is slightly convex and is proceeds laterally. The prothoracic pleurites coalesce with the tergum. The separation of the epimeron and episternum is indistinctly developed and the epimeron runs antero-ventrally and forms the praecoxale. The sternite of the prothorax is broadly developed and the sternal pit seems to be recessed. The majority of the prothoracic sternite is formed by the basisternum 1, the cranial part of the base of the coxae. The small furcasternum 1, located posterior to the latter, separates the left and right coxa. It is followed by the intersternite, which forms the transition to the mesosternite. The cranialmost part of the pterthoracic tergite is acrotergite 2 followed posteriorly by the dorsal carina and enclosed laterally by the mesostigmata. The acrotergite 2 is medially punctuated with the pit of the tergal apophysis 2. The small prescutum 2 is located cranial to the pit of the tergal apophysis 3. The small prescutum is connected to the humeral plate 2. The humeral plates are axillary sclerites at the wing base. The center of the mesothorax forms the nearly rhomboidal scutum 2, which is connected with the radius and axillary plate 2 by their lateral branches. The scutelum 2 is located posterior to the scutum 2. The scutelum is the origin of the axillary cords, which run latero-caudally and connect the scutelum to the anal veins. The scutelum 2 is followed by the large and nearly trapezoidal postnotum 2. The structures of the metathoracic part of the pterothorax are basically and functionally identical to those of the described mesothoracic part. The prescutum 3 is overlaid by the posterior edge of the postnotum 2. Compared to the postnotum 2, the postnotum 3 is only weakly

developed. The pleurites of the pterothorax are tilted posterior and are one of the most modified characters in Odonata. The collar carina, antelar carina and sinus as well as the apical carina are only slightly developed and located on the broad episternum 2. The two-branched wing process 2 is located dorsally to the distinct mesopleural suture and articulates with the wing base. The metathoracic spiracle is located posterior to the end of the interpleural suture. It also demarcates the epimeron 2 from the episternum 3. This suture ends more or less at one third of the dorso-ventral expansion of the pterothorax. The wing process 3 is similar in shape but distinctly smaller than wing process 2. The pterothoracic sternites are difficult to study because of the legs, which block the view of the sternum. It seems to display the same characteristics as in *E. superstes* (cf. Asahina 1954).

Thorax colouration is characterized by two yellow stripes; the mesepisternal and the metaepisternal one. The rest of the thorax is dark brown with a ventral gradient to lighter brown. The posterior part of the epimeron 3, which is a lighter shade of brown as well (Supl. 2 & 3).

The legs show a colour gradient. The coxa, trochanter and 2/3 of the femur are lighter brown than the rest of the femur. The tibia and tarsi are nearly black. The base of the coxa is dark brown as well. The femur is cylindrical and shows two-rowed bars of small spines ventrally. Characteristic for *Epiophlebia* is an assemblage of semicircular tubercles on the dorsal side of the femur. The nearly rectangular tibia provide two rows of strong and long spines running laterally on the flat dorsal side.

The wing shows differences, but even within one individual; therefore, the usage of this character system is rather questionable.

Abdominal Characters

The abdomen of *E. laidlawi* is more or less cylindrical and composed of ten segments. segments 3 to 7 have almost the same length and shape and are laterally depressed, whereas segment 7 broadens towards segment 8. The colouration of the abdomen is brownish with a dorso-ventral colour gradient to nearly black towards the tip of tergite. segments 2 to 8 show a more or less yellow triangular apical spot dorsally. segment 9 shows a slightly shapeless often very dark yellow spot at the same location. Laterally the segments 2 to 8 show lighter brownish spots that appear almost transparent (Suppl. XXX).

Generally, the male copulatory organ is located at abdominal segments 2 and 3. The secondary male genitalia start at the anterior-most part with a more or less shapeless plate, the lamina anterior. This lamina anterior has a convex elevation in the center. The hamuli anterior are bump-like and quite short. Given the undissected condition of the

specimens, the more internal structures are difficult to distinguish, but seem to show the same characteristics as *E. superstes* (cf. Asahina 1954). The most characteristic part of the secondary male genitalia is the hamulus posterior, which is anchor-like. The primary male genitalia are located at sternite 9 and are partly covered by the surrounding valvues. The nymphal paraprocts are nearly hairless and form two equal sides of an isosceles triangle. The other structures of the male abdomen are comparable to *E. superstes* (cf. Asahina 1954).

The female genitalia are located at abdominal segments 8 and 9. The ovipositor system is comparably undeveloped for Odonata and resembles that of *E. superstes* (cf. Asahina 1954), except that the process of sternite 8 is like a blunt hook.

The caudal appendages of abdominal segment 10 show the same characteristics as *E. superstes* (cf. Asahina 1954). The superior appendage and cercus are short and more or less rudimentary with notched apical parts. The inferior appendage, however, is strongly developed.

Discussion

Acknowledgements

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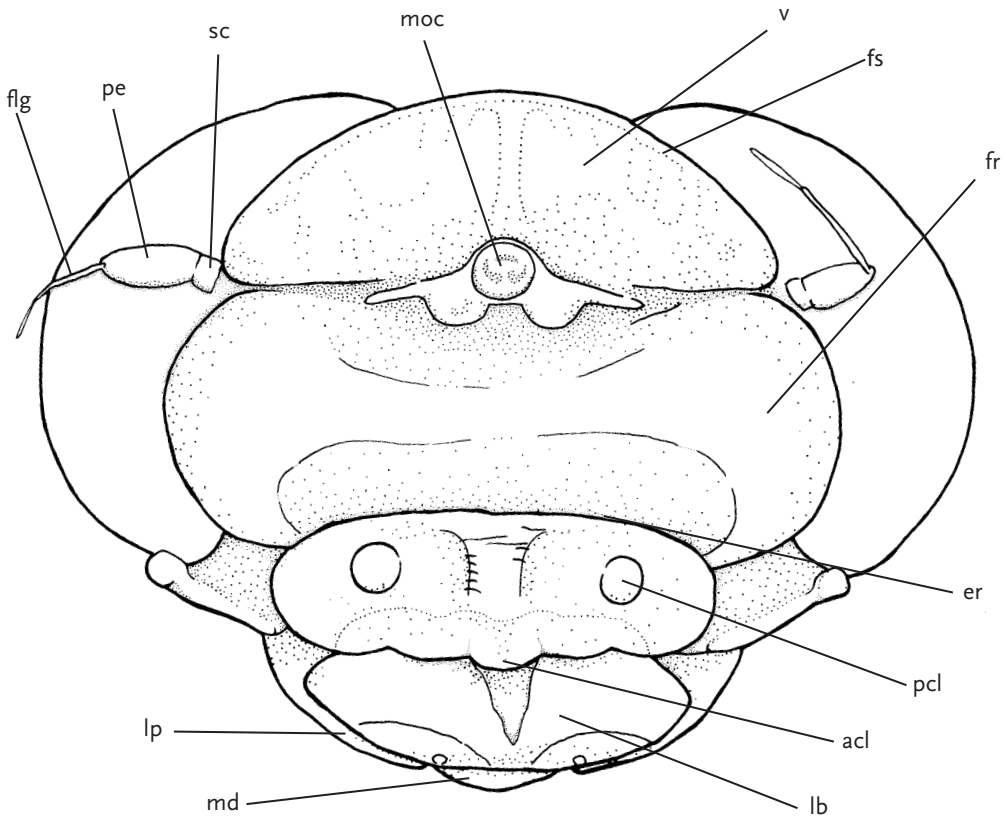

Figure 1: Drawing ot the head of *Epiophlebia laidlawi* frontal view.
Abbreviations: acl - anteclypeus, er - epistomal ridge, flg - flagellum, fr - frons, fs - frontal sutur, lb - labium, lp - labial palp, md - mandible, moc - median ocellus, pcl - postclypeus, pe - pedicel, sc - scape, v - vertex.

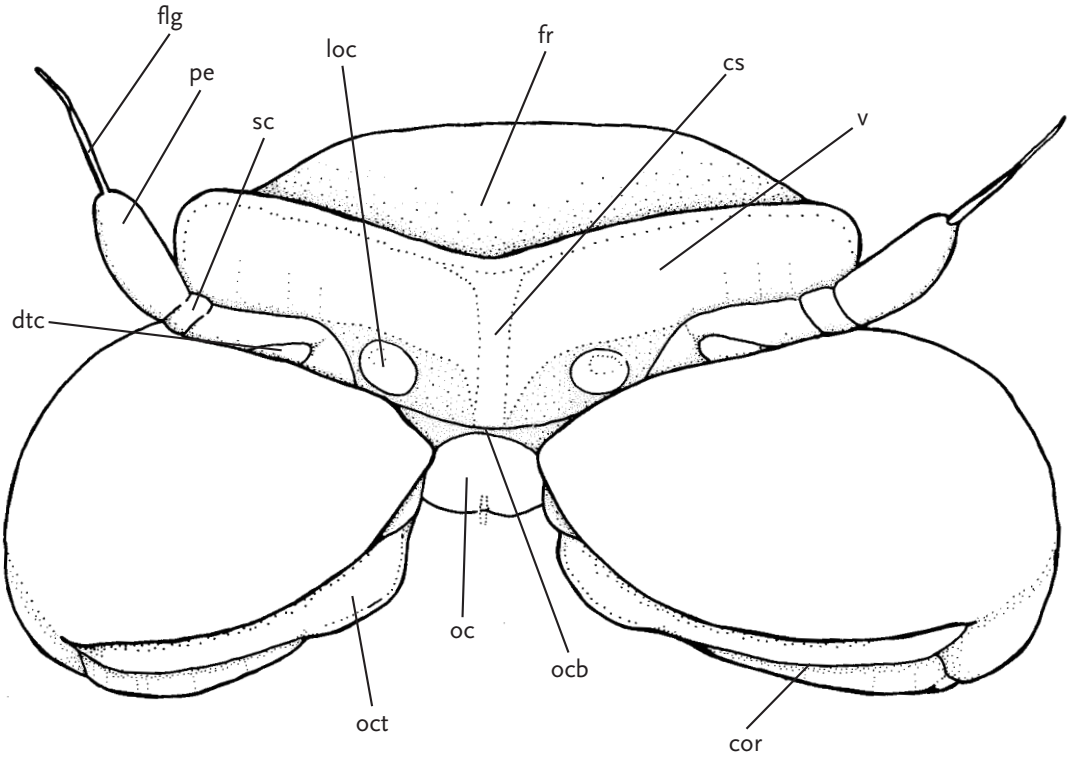

Figure 2: Drawing ot the head of *Epiophlebia laidlawi* dorsal view.
Abbreviations: cor - circumocular ridge, cs - coronal sulcus, dtc - dorsal tentorial cavity, flg - flagellum, fr - frons, loc - lateral ocellus, oc - occiput (dorsal part), ocb - ocipital bar, oct - ocipital tubercle, pe - pedicel, sc - scape, v - vertex.

Supplement 1: *Epiophlebia laidlawi*, adult male, dorsal overview.

Supplement 2: *Epiophlebia laidlawi*, adult male, dorsal view of head and thorax.

Supplement 3: *Epiophlebia laidlawi*, adult male, lateral view of head and thorax.

Supplement 4: *Epiophlebia laidlawi*, adult male, frontal view of head and thorax.

8. Final Discussion and Conclusions

Insect flight is the key factor for the success of this most diverse group of organisms. The insect flight apparatus, its development, evolution and the ground pattern are however only poorly understood. The overall aim of the present studies is therefore to investigate evolution of the thorax of Odonata using modern morphological techniques to provide detailed character analysis. The obtained morphological data are used for establishing a homology hypothesis for the thorax muscle setup of Odonata and Neoptera and estimating a musculature set for a generalized odonatan thorax, which presents all recently known thorax muscles for Odonata. The phylogeography and species status of one of the most enigmatic odonatans – *Epiophlebia* – will furthermore be elucidated by exploiting the advantages of DNA analysis.

To avoid undue repetition of discussions presented in previous chapters, I will focus on the key evolutionary features of the odonatan thorax, especially the thorax musculature, and explain further coherences and summaries in the following.

8.1. *Epiophlebia*

First genetic study of 'all' *Epiophlebia* species – unexpected homogeneity of DNA – one of first description of post-Ice Age relicts in Asia – taxonomic review to clear species status

Epiophlebia species seems to represent the most ancestral character distribution of all known Odonata (Blanke et al. 2013a). Its conspicuous mixture of zygopteran and anisopteran characters (Asahina 1954, Büsse et al. 2012) placed *Epiophlebia* outside the two major groups – damselflies and dragonflies (e.g. Bybee et al. 2008). Still more, characters of recent *Epiophlebia* are present in Zygoptera and the stem-group of Epiprocta, but neither in the stem-group of Anisoptera and nor in the crown-group of Anisoptera. Phylogenetically, it is one of the closest representatives, placed at the split of Anisoptera and Zygoptera – of all known Odonata, including fossils (e.g. Lohmann 1996).

This unique phylogenetic position and character combination, (Blanke et al. 2013a), disjunct distribution (Büsse et al. 2012) and lack of knowledge of all *Epiophlebia* species with the exception of *Epiophlebia superstes* prompted closer investigation of these enigmatic Odonata. *Epiophlebia* comprises only four described species in the extant fauna – *E. superstes* (Sélys, 1889) from Japan, *E. laidlawi* Tillyard, 1921 from the Himalayas, and two very recently described species from China *E. sinensis* Li & Nel, 2011 and *E. diana* Carle, 2012 (Figure 13).

8.1.1. Molecular and Phylogeographic Analysis of *Epiophlebia*

The genetic study of *Epiophlebia* (Büsse et al. 2012) was designed to test the sister

group relationship of *E. superstes* and *E. laidlawi*, which was proposed by Asahina (1961, 1963). All recent studies deduce this sister group relationship (e.g. Brockhaus & Hartmann 2009, Li et al. 2011, Carle 2012, Blanke et al. 2013a), but a reinvestigation and taxonomic review of Asahina's (1961, 1963) non-comprehensive studies of *E. laidlawi* is absent.

Our findings are, however, greatly surprising. Three species for which DNA exchange can be ruled out on the basis of sheer distance combined with a stenoecious life cycle indicate extreme genetic similarity. The investigated conservative sequences of 28S rRNA and 18S rRNA and the more variable sequences, ITS1, ITS2 and CO2 show very little or no differences between *E. superstes*, *E. laidlawi* and *E. sinensis*. To quantify: The investigated sequences of 18S rRNA genes (240 bp) and 28S rRNA genes (1002 bp) did not show any differences between *E. laidlawi* and *E. superstes*. For CO2 (265 bp) a single difference relative to the *E. superstes* reference sequence was found in all specimens including the Japanese control specimen (cf. Büsse et al. 2012). The fragment of ITS2 (265 bp) shows a maximum of four differences, and ITS1 shows an absolute maximum of 11 differences between *E. superstes* and *E. laidlawi*; however, six of the maximum differences of 11 point mutations could be traced to one insertion event (Büsse et al. 2012). Such a small quantity of differences in DNA sequences normally occurs between allopatric populations of one species; e.g. in the population of *Calopteryx haemorrhoidalis* (Vander Linden, 1825) in Spain and Tunisia (Büsse et al. 2012).

This result raises the question of *E. laidlawi*'s species status. A DNA exchange between the populations seems to be totally impossible at the present time. The DNA similarities suggest *E. superstes*, *E. laidlawi* and *E. sinensis* had a common ancestor with the split dating back to only 20,000 years ago. By contrast, the former biogeographic hypothesis by Brockhaus & Hartmann (2009) suggests the *Epiophlebia* species originated during the Jurassic on the rising continent of Eurasia (approximately 200-145 MYA).

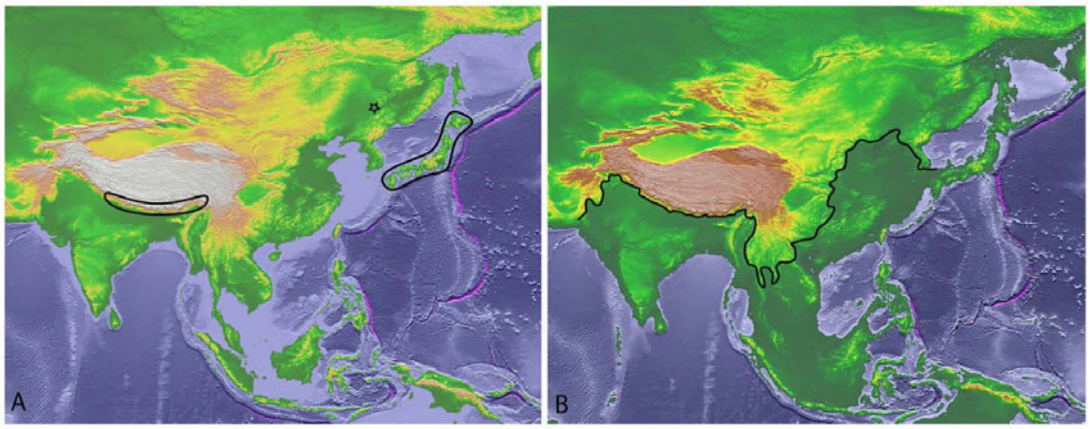

Figure 13 - Distribution of *Epiophlebia* . **A** - Known present distribution, the star marks the type locality of *E. sinensis* Li & Nel, 2011. **B** - Approximate coastline during the Würm glacial period with land bridges between Japan and the mainland. The black line marks a possible northern boundary of the range of *Epiophlebia* during this time. (After Büsse et al. 2012).

The newly presented biogeographic estimation by Büsse et al. (2012) is founded on accepted DNA mutation rates (e.g. Friedrich & Tautz 1997, Ho et al. 2011).

Twenty-thousand years ago during the Würm ice age, the Sino-Japanese floristic region (Ikeda & Ohba 1998) formed the last possible habitat connection by a land bridge to Hokkaido (Clark et al. 2009). According to some authors (Millien-Segota 1973, Tushingham & Peltier 1993, Parra & Jaeger 1999) a land bridge also existed between Kyushu and the Korean peninsula during this time (Figure 13). These assumptions are underscored by the fact that all known *Epiophlebia* species were distributed solely in glacial refuges as defined by De Lattin (1956), the only route of retreat at the warming phase that was starting (unpublished data Lohmann & Büsse).

Combining these biogeographic assumptions with the DNA mutation rates mentioned indicates the DNA similarities discovered are self-explanatory while challenging our comprehension of a species.
There are at least 24 different species concepts (Mayden 1997), and perhaps even more (Queiroz 2007). These concepts reflect various biological opinions and needs of many different sub-disciplines with a high frequency of heterogeneous characters (e.g. Queiroz 2005). For example, the biological species concept deals with reproductive entity (Mayr 1942, 1982) whereas the ecological species concept assumes an explicit niche (Valen 1976, Andersson 1990), and the phylogenetic species concept deals with recognisability (Nixon & Cracraft 1983, Nixon & Wheeler 1990,) or monophyly (Rosen 1979, Donoghue 1985).

The currently debated problem has led us to 'Integrative Taxonomy' (Padial et al. 2010, Schlick-Steiner et al. 2010), which is known for combining several kinds of phenotypic or/and genotypic data (Haszprunar 2011). Although homogeny of genetic data in *Epiophlebia* suggests one allopatric population, the morphology shows distinct differences. The shape of the secondary male genitalia of *E. superstes* and *E. laidlawi*, in particular, function according to the lock-and-key principle and can act as a reproductive isolation factor. These morphological investigations answer the question of the species status as demonstrated in the next chapter.

8.1.2. Morphology of *Epiophlebia laidlawi*

The morphological investigation of *E. laidlawi* (Asahina 1961) nymphs and adults (Asahina 1963) suggests a sister group relationship between *E. laidlawi* and *E. superstes*. The studies show less discriminating characters but nevertheless reveal a few that are distinctive. The aim of my present study (Büsse in prep.) is a review of *E. laidlawi* to elucidate the morphological differences between *E. laidlawi* and *E. superstes*. In addition, some insights on the species status and validity of *E. sinensis* and especially *E. diana* should have been included but are incomplete at this point. They will, however, be discussed briefly a as a supplement to the following.

Epiophlebia laidlawi shows some unique morphological characters (cf. Büsse in prep.):

- The vertex is u-shaped in *E. superstes* and v-shaped in *E. laidlawi*.
- The antefrons is proximally keeled in *E. laidlawi* and padded in *E. superstes*.
- The apical occipital is convex in *E. superstes* and steeply declining to the foramen in *E. laidlawi*.
- The colouration is black with more yellow stripes in *E. superstes* and more brownish with fewer yellow stripes in *E. laidlawi*.
- The abdominal sternite 8 is distinctly sharper and less rounded in *E. superstes* relative to *E. laidlawi*.
- The hammulus posterior is anchor-like in *E. laidlawi* and looks like a scorpion's stinger in *E. superstes*.

To sum up, *Epiophlebia laidlawi* is a morphologically well-defined species and not an allopatric population of *E. superstes*, as suggested in Büsse et al. (2012). The secondary male genitalia, in particular, can prevent cross-fertilization based on the lock-and-key principle. Furthermore, the different abdominal coloration is indispensable for female choice (e.g. Corbet 1999). Both these characters, can act as reproductive isolation factors and amply address the question of species status, as mentioned above.
The same might also be true for E*piophlebia sinensis*; the most prominent characters are a rufism – reddish brown colloration – on the last segments of the abdomen (cf. Li et al. 2011). Yet *Epiophlebia diana* is a contradiction. The already sparse and debatable characters that should distinguish *E. diana* from *E. laidlawi* (Carle 2012) are questionable. The scape and pedicle should be as long as the first flagellar in *E. diana* and longer than the first flagellar in *E. laidlawi*. In fact *E. laidlawi* displays both character states. The prementum in *E. diana* has strongly sinuous margins whereas in *E. laidlawi* this margins are only slightly developed. *E. laidlawi* specimens' premental margins vary substantially, but are always distinctly prominent. The same is true for the abdominal lobes, which protrude on segment nine and show high variation in *E. laidlawi*. The characters are questionable because *E. laidlawi* shows the same character stages or because of the significance of numerical characters at a sample size of just two. Therefore, *Epiophlebia diana* Carle, 2012 might be declared as a junior synonym of *Epiophlebia laidlawi* Tillyard, 1921.

8.2. Universal Analysis System for Taxonomic Identification

Genetic analysis of degraded DNA – universal species identification – access to the biodiversity of museum collections – highly discriminating human DNA allows forensic probes

The universal analysis system for taxonomic identification, even of degraded DNA – of formol fixated samples or ancient DNA (aDNA) for example – was a finding of the *Epiophlebia* study by Büsse et al. (2012).

The specimens of *Epiophlebia laidlawi* were fixed for morphological studies. The most common morphological fixation methods, among them Dubosq-Brasil fixative, FAE or Carnoys (cf. Romeis 1987), include formol or other DNA degrading ingredients. Therefore, a special approach, standardised for application in aDNA studies (Hummel 2003), was used to analyse the strongly segmented DNA strands. This comprised detection of highly variable regions in the targeted sequence and use of self-designed primer pairs. During this process, one highly variable region (S4) on the 28S rRNA gene we targeted turned out to be useful for taxonomic identification, not just in Odonata but for all other insects as well (Grumbkow et al. subm.).

This analysis system can be useful in various biological research fields such as biodiversity research, forensic entomology or taxonomy. The recent standard methods of species identification (e.g. Hebert et al. 2003b, Gomez et al. 2007) require expert knowledge and are often time consuming, whereas identification via CO1-barcoding can be unreliable for certain taxa or poorly preserved specimens (Figure 14; e.g. Whitworth et al. 2007, Herrera et al. 2010, Smith et al. 2012). The analysis system presented here allows species identification of insect specimens or even parts and is applicable for the type of degraded DNA found in museum specimens or in a forensic context. The primers are designed to discriminate against human DNA, making the method especially useful for contexts where contamination with exogenous DNA is likely.

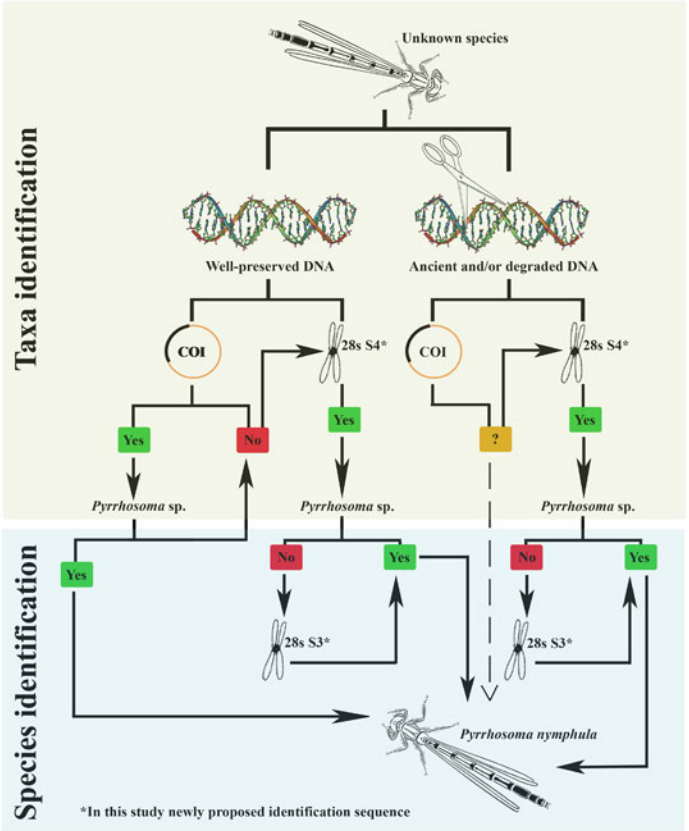

Figure 14 - Diagram of proposed procedure for identifying insect species (S4-28S).

The system was tested on carefully selected species covering all insect groups historically ranked as orders (cf. von Grumbkow et al. subm.).
In most cases, just one short sequence enabled reliable species-level identification of the specimen investigated. It can therefore be concluded that the system presented here is a useful tool for species identification and a valuable addition to CO1-barcoding - Figure 14 shows an overview of the our S4-methods and how they compare with CO1-barcoding.

8.3. The Flight Apparatus of Odonata and Homologies with Neoptera

The first complete homologization scheme of Odonata and Neoptera thorax musculature – intermediate Ephemeroptera sclerites corroborate Palaeoptera hypothesis – wing base sclerites' homologies were proofed and extended – eleven newly described thorax muscles – a set of apomorphies supports the assumption of a highly-derived Odonata thorax

My thesis study of the thorax of Odonata is meant to provide a basis for homology hypothesis of Odonata and Neoptera flight apparatus. It provides the first comprehensive homology hypothesis for the thorax musculature (Büsse et al. 2013, Büsse & Hörnschemeyer subm.). Furthermore, it completes and reiterates the present homology hypothesis of wing base structures (Genet et al. subm.). Taken as a complete unit, it fills some important knowledge gaps of the morphology of the Odonata flight apparatus and provides the first comprehensive homology hypothesis of thorax musculature in Neoptera.

Several publications address the Odonata flight apparatus structures (Chao 1953, Asahina 1954, Tannert 1958, Russenberger 1960, Pfau 1986), the aerodynamics of odonatan flight (Pfau 1986, Dudley 1990, Brodsky 1994), mechanics (Hatch 1966) and function of the flight musculature, mechanoreceptors of the wing (Pfau 1986) and the complexity of the wing venation (Bechly 1996) almost exclusively in Anisoptera. The thorax musculature of adult dragonflies (e.g. Clark 1940, Hatch 1966, Pfau 1986) and also of *Epiophlebia superstes* (Asahina 1954) is comparatively well-investigated. Far less investigation has been done of the thorax of adult damselflies (Asahina 1954, Ninomiya and Yoshizawa 2009). What is more, Odonata nymph thorax musculature has only begun to receive a smattering of attention in the literature so far (Whedon 1929, Maloeuf 1935, Asahina 1954).

The homology hypothesis of the Neoptera wing base structures and flight musculature (Hörnschemeyer 2002, Willkommen & Hörnschemeyer 2007, Willkommen 2008, Friedrich & Beutel 2008) and Ephemeroptera (Yoshizawa & Ninomiya 2007, Willkommen 2008) are most widely accepted. However, the hypotheses on the homologies between Odonata and the remaining Pterygota are still being discussed (Pfau 1986, Yoshizawa & Ninomiya 2007, Willkommen 2008, Ninomiya & Yoshizawa 2009).

The aim of comprehensive comparative investigation of adult Zygoptera (Büsse et al. 2013, Genet et al. subm.) and Anisoptera nymph (Büsse & Hörnschemeyer subm.) flight apparatus is to identify variability among the Odonata, to underscore the present hypothesis of wing base structures (cf. Ninomiya & Yoshizawa 2009) and establish homology hypotheses for the thoracic musculature of Odonata and Neoptera.

8.3.1. Wing Base Structures

The central aim of this study is to contribute to the understanding of the highly derived odonatan flight apparatus. In contrast to recent, dominant opinions (e.g. Matsuda 1970) that the wing articulation of Odonata and Ephemeroptera represents a plesiomorphic state, more recent investigations conclude that the flight apparatus of these taxa are highly modified, i.e. derived. We furthermore clarify the homologies of the skeletal elements of Odonata and Neoptera pterothorax. Contrary to all remaining Pterygota, the flight musculature of Odonata actuates the wings directly (Snodgrass 1935). This distinctly influences the structures of Odonata wing articulation (Tannert 1958, Pfau 1986). Substantial differences between the flight apparatus of Odonata and other Pterygota make the homologization of the sclerites and thorax muscles difficult. Büsse et al. (2013), however, presents an initial consistent and comprehensive hypothesis for the homologization of the pterothoracic musculature of Neoptera and Odonata. A homologization of the thoracic and wing base sclerites between Odonata and Neoptera represents a new set of characters and thus supports future comparative work and phylogenetic analysis, using these characters of the flight apparatus throughout all winged insects. This will contribute to understanding the early evolution of pterygote insects and their basal phylogenetic relationships.

Another goal of this study, however, is to use muscle attachment points on the wing base sclerites as additional homology criteria. For this purpose, the information about thorax muscle attachment points published in Büsse et al. (2013) was used as a point of reference.

The homologization of key structures of the flight apparatus of Odonata and Neoptera is fairly straightforward. The study recently presented in Büsse et al. (2013) showed adult Zygoptera thorax muscle setup and suggested a homologization scheme for the pterothoracic musculature of Odonata and Neoptera. Including the information presented here, the most important sclerites can also be homologized: the distal costal plate of Odonata is homologous to the neopteran humeral plate. This homologization was also underscored by Ninomiya & Yoshizawa‘s (2009) investigation. Different homologization schemes have also been published regarding Neoptera‘s first, second and third axillary sclerites (Willkommen & Hörnschemeyer 2007, Willkommen 2008, Ninomiya & Yoshizawa 2009). Our study supports Ninomiya & Yoshizawa (2009) by suggesting homology of Odonata‘s central axillary plate to the axillary sclerites one to three of Neoptera, including the median plates. Additionally, the Odonata‘s proximal costal plate is homologous to the neopteran basalare.

Odonata's posterior pleural process and posterior tergal lever are homologous to neopteran subalare.

Odonata's proximal costal plate is part of the dorsal wing base, therefore a tergal sclerite; whereas Neoptera's basalare is located at the pleura (Snodgrass 1935). The Ephemeroptera, by contrast, represents an intermediate state. Ephemeroptera's basalare is a brace like sclerite, which is stretched from the antero-dorsal parts of the wing base to the dorsal pleurum (Willkommen & Hörnschemeyer 2007, Willkommen 2008). Homologization is therefore most likely.

Our findings clearly indicate Odonata's flight apparatus is a highly derived version of what was present in the last common ancestor of Pterygota (cf. Chapter 8.2.3). Furthermore, the intermediate state of the basalare of Ephemeroptera in comparison to the condition of the basalare in Neoptera and the axillary plate in Odonata support the Palaeoptera hypothesis (Genet et al. subm.) – the sister group relationship of Ephemeroptera and Odonata (e.g. Blanke et al. 2012a).

8.3.2. Thorax Musculature

Studies of adult Zygoptera (Büsse et al. 2013) and nymphal Anisoptera (Büsse & Hörnschemeyer subm.) comprise the investigation of Odonata thorax musculature. Knowledge of odonatan thorax morphology reflects distinct deficits for Zygoptera and nymphs in general. We focused our comparative investigation on these areas.

Skeletal elements and thorax musculature as mentioned in Genet et al. (submitted) interact to move the wings. The studies' central aim is to investigate a homology hypothesis of thorax muscles found in Odonata and Neoptera. In addition, flight musculature could also be used for homologization of the wing base sclerites (cf. Genet et al. subm.).

The results identify differences in the Zygoptera and Anisoptera and contribute to greater understanding of odonatan thorax musculature. A homologization scheme for the thorax musculature with a generalized Neoptera thorax (Friedrich & Beutel 2008) was also presented in Büsse et al (2013) and completed in the study by Büsse & Hörnschemeyer (subm.).

An Odonata pterothorax is made up of, according to Asahina (1954), 51 thorax muscles in general and 42 muscles for adult Zygoptera. Of these muscles, 19 belong to the mesothorax in Zygoptera; Muscle 35 and Muscle 37 are lacking in adults (Asahina 1954). The remaining 23 muscles belong to the metathorax. In this case, Muscle 47, Muscle 57 and Muscle 60 are not present in adult Zygoptera (Asahina 1954). The 42 muscles identified by Asahina (1954) could be confirmed. In addition, two new odonatan thorax muscles were found (cf. Maloeuf 1935, Asahina 1954, Tannert 1958, Pfau 1986). These tergo-pleural muscles, IItpm2 and IIItpm2, are short, slender and run from the dorsal part of the pleural bar to the median semi-detached scutal plate. They run parallel to

IItpm4 and IIItmp4 with the same position and direction. A reinforcing function is therefore assumed. In Zygoptera, these muscles (IItpm2 and IIItpm2) are plesiomorphic and their reduction represents an apomorphy of Epiprocta. Homologizing the mesothorax and metathorax musculature of adult Zygoptera with Neoptera was surprisingly straightforward (Büsse et al. 2013). Though a couple of observed origin and insertion points differ from Friedrich & Beutel (2008), neither muscle direction nor function was influenced considerably (cf. Büsse et al 2013). We were also able to identify indirect flight muscles (Kéler 1963) in the odonatan pterothorax. As mentioned above, the flight musculature of Odonata actuates the wings directly, unlike all other Pterygota (Snodgrass 1935). The muscles IIdlm1, IIIdlm1 and IIIdlm2 originate at the tergal apophysis and are homologous with the dorsal longitudinal muscles of the neopteran pterothorax (Pfau 1986, Büsse et al. 2013). The longitudinal muscles run between the phragmata in the ground pattern of the Neoptera (Chapman 1998). The insertion point of muscle IIdlm1, for example, is at the postnotum's caudal edge, i.e. at the caudal end of the second thorax segment. This is equivalent to the position of the phragmata (respectively pseudophragmata) in Neoptera (Matsuda 1970) and supports the proposed homologization and identification as indirect flight muscles (Büsse et al. 2013). We can show here that Odonata use direct and indirect actuation for producing the wing beat (Büsse et al. 2013). This distinctly indicates that the thorax of Odonata is a highly derived character system. Reduction of the indirect flight muscles and direct actuation of the wing as apomorphies underscore the elevated level of derivation.

Maloeuf (1935) remarked that Odonata nymphs have a greater number of leg and neck muscles than adults and that these tend to be sturdier. At any rate, the number and type of muscles differ between nymph and adult Odonata (e.g. Maloeuf 1935, Asahina 1954). During ontogenesis, the musculature is reformed, transformed or reduced (Whedon 1929, Maloeuf 1935). This seems to be an apomorphy due to the fact that starting with the first instars, other hemimetabolous insects exhibit a full complement of musculature (e.g. Wittig 1955, Willkommen & Hörnschemeyer 2007). Büsse and Hörnschemeyer (subm.) identify 71 muscles (e.g. Asahina (1954) mentioned 51 in general for Odonata), 19 prothorax muscles, 26 mesothorax muscles (e.g. Büsse et al. 2013 described 19 for adult Zygoptera), and 27 metathorax muscles (e.g. Büsse et al. 2013 described 23 for adult Zygoptera) for Anisoptera nymphs. This includes nine previously undescribed muscles for Odonata (Ispm1, IIscm1 (IIIscm1), IIscm2 (IIIscm2), IIscm8, IItpm3 (IIItpm3) and IIIscm4), as well as seven muscles (Ipcm9, Itpm7 – Itpm11, IIscm8), which have no homologue in the neopteran thorax.

Odonata shows a highly reduced thorax muscle setup (apomorphy) compared to the pterygote ground pattern and to Neoptera, latter having 92 more thorax muscles than Odonata (cf. Friedrich & Beutel 2008). The seven muscles of Odonata that have no homologue in the neopteran thorax (mentioned above) represent a plesiomorphic state. These Odonata thorax muscles are unique for a recent group of pterygote insects.

As mentioned in Genet et al. (subm.) and Büsse et al. (2013), homologization of the odonatan musculature with Neoptera was straightforward; the same is true for the homologies of the nymphal thorax (Büsse & Hörnschemeyer subm.). Furthermore, we found an interesting evolutionary trade-off relating to the newly described odonatan muscle Ipcm9, that shows counterparts in the mesothorax (IIpcm5) and metathorax (IIIpcm5) of Neoptera (cf. Friedrich & Beutel 2008). However, IIpcm5 and IIIpcm5 are not present in Odonata. The pterygote ground pattern shows Ipcm9, IIpcm5 and IIIpcm5 as prothorax, mesothorax and metathorax counterparts. Within the Odonata, IIpcm5 and IIIpcm5 were reduced. On the other hand, Ipcm9 were reduced in Neoptera. Therefore, the reduction of IIpcm5 (IIIpcm5) is an apomorphy of Odonata.

In the following, I accordingly present a generalized odonatan thorax that comprises all the muscles known so far for Odonata and initial ideas for the ground plan of a pterygote ancestor.

8.3.3. Generalized Odonata Thorax and the Ground Pattern of Pterygote Insects

Generalized Odonata thorax that includes all known odonatan muscles – discussion of important thorax character and phylogenetic relevance – first insights into the thorax of a stem-species representative of pterygote insects

The generalized odonatan thorax (Figure 15-17) shows all the muscles that have been found in Odonata to-date (Supplement 1). It compiles all the results of this thesis (Büsse et al. 2013, Büsse & Hörnschemeyer subm.) and is completed by four muscles located independently by both Asahina (1954) and Maloeuf (1935) only. For simplicity's sake and ease of comparison to Neoptera in particular, the generalized odonatan thorax is shaped like a nymphal thorax, which resembles the neopteran thorax. In order to present an overview, all structures, attachment points and directions have been simplified.
It includes all muscles found homologous to Neoptera (Büsse et al. 2013, Büsse & Hörnschemeyer subm.) and the newly described Odonata muscles with no homologies to neopteran thorax (cf. Supplement 2).

Asahina (1954) and Maloeuf (1935) described four muscles in the Odonata thorax that had not been found in the presented studies. These are: M6, originating in the prothorax at the cervix close to the postoccipital ridge and inserting at a sclerotization in the dorso-lateral part of the neck membrane, M8, a prothoracic muscle that originates at the body of the tentorium and inserts at the cervical sclerite, and the mesothoracal M24 and its metathoracal counterpart M47, originating at the tergo-pleural ridge and inserts at the dorsal area of the episternum (Maloeuf 1935, Asahina 1954). Possible homologues in the neopteran thorax are Musculus pronoto-cervicalis lateralis Idvm4 for M6 (Figure 15), Musculus prophragma-mesanepisternalis IItpm1 respectively Musculus mesophragma-metanepisternalis IIItpm1 for M24 (47) (Figure 16) and no

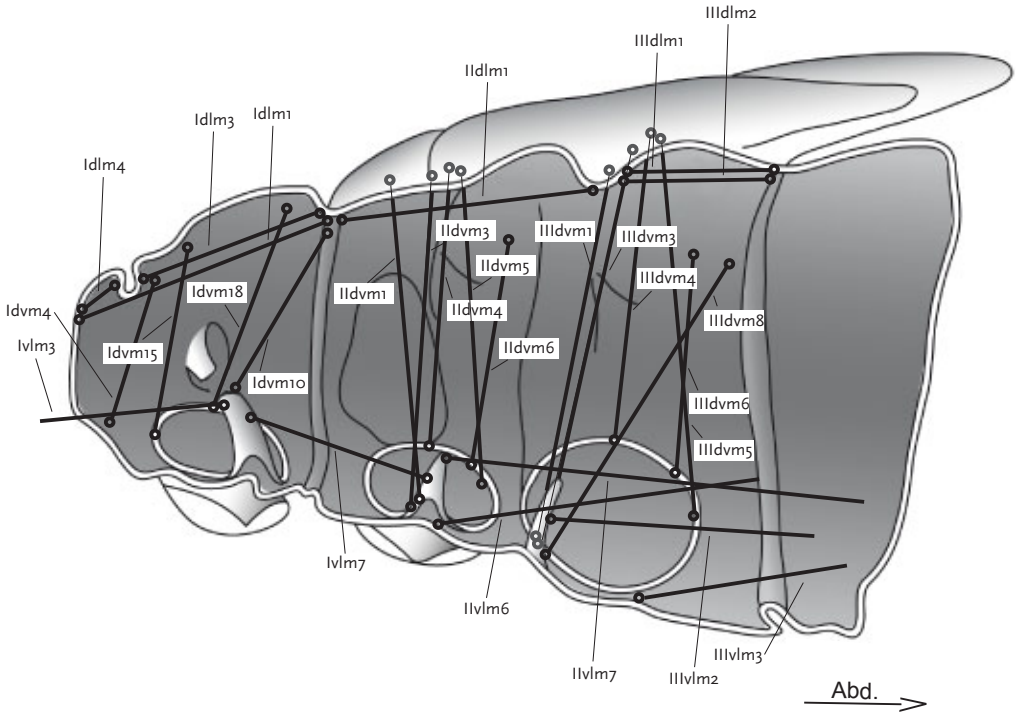

Figure 15 - Generalized Odonata thorax, showing the dorso-ventral, ventro-lateral and dorso- longitudinal musculature of a hypothetical odonatan ground pattern. Abbreviations: Abd – abdominal segment, dlm – dorso-longitudinal musculature, dvm – dorso-ventral musculature, vlm – ventro-lateral musculature.

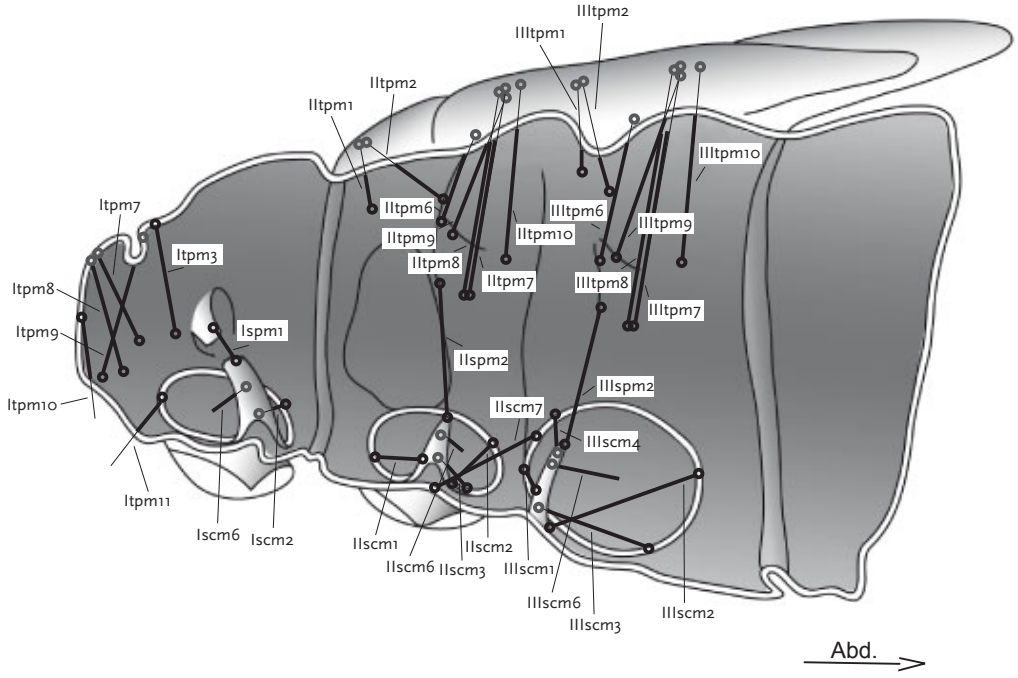

Figure 16 - Generalized Odonata thorax, showing the sterno-coxal, sterno-pleural and tergo- pleural musculature of a hypothetical odonatan ground pattern. Abbreviations: Abd – abdominal segment scm – sterno-coxal musculature, spm – sterno- pleural musculature, tpm – tergo-pleural musculature.

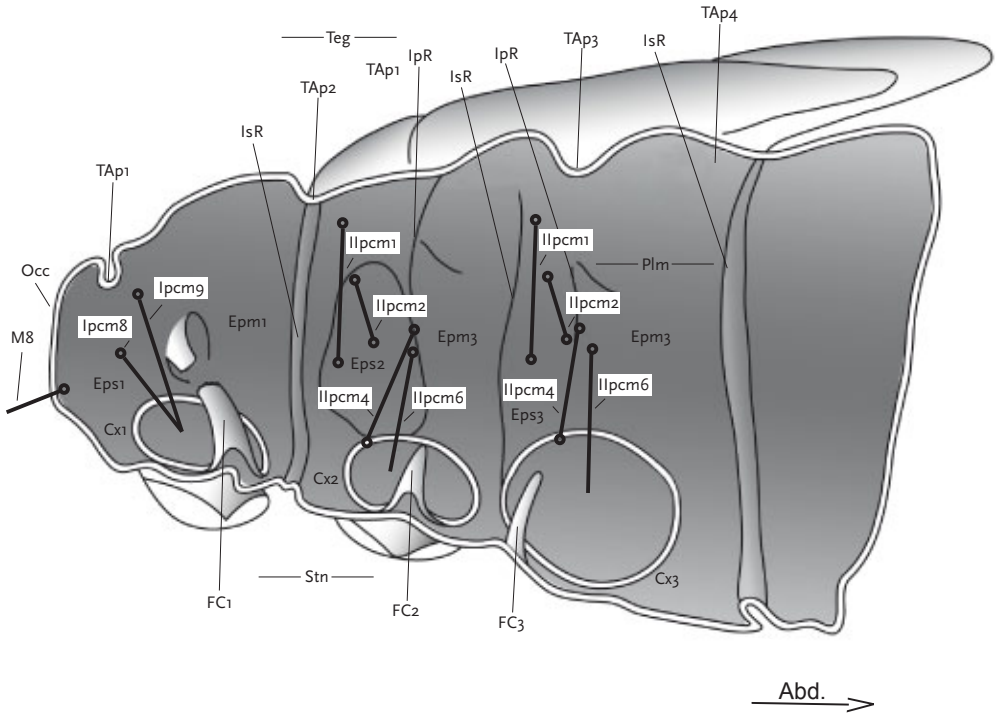

Figure 17 - Generalized Odonata thorax, showing the pleuro-coxal musculature and important skeletal elements of a hypothetical odonatan ground pattern. Abbreviations: Abd – abdominal segment, Cx – coxa, Epm – epimeron, Eps – episternum, FC – furca, IpR – interpleural ridge, IsR – intersegmental ridge, Occ – occiput, pcm – pleuro-coxal musculature, Plm – pleurum, Stn – sternum, TAp – tergal Apophysis, Teg – tergum.

possible homologue to M8 (Figure 17). The latter might represent an apomorphy for Odonata.

The aim of the generalized odonatan thorax (Figure 15-17) is to gain clear understanding of Odonata's muscle setup. It also represents an initial attempt to develop a hypothetical odonatan ground pattern of a stem-group representative.

Because of the high amount of newly described muscles found in the Anisoptera nymphs in particular, (Büsse & Hörnschemeyer subm.) it might be advisable to reinvestigate the Zygoptera nymphs and Epiprocta adults [Anisoptera+*Epiophlebia*] to complete the generalized Odonata thorax.

Additionally, an initial attempt at developing the pterygote ground pattern could include all known muscles of Neoptera and all described muscles of Odonata. This is because starting with a ground pattern of Dicondylia (apterygote insect) means a high numbered muscle setup is most likely. Zygentoma and even Archaeognatha show an almost uncountable number of single stranded muscles in their thorax (e.g. Barlet 1953, 1954, 1967). Furthermore, the indirect flight mechanism of Neoptera involves a higher number of muscles compared to the direct mechanism of Odonata (cf. Chapter

8.3.2). The neopteran condition presents the plesiomorphic condition.

The most important results of all the thorax studies in my thesis (Büsse et al. 2013, Genet et al. subm., Büsse & Hörnschemeyer subm.) and the reconstructed pterygote ground pattern can be mapped on a phylogenetic tree (Figure 18). The related groups' thorax apomorphies and plesiomorphies underscore the recent hypothesis. The mapped characters (cf. Figure 18) will be discussed as follows:

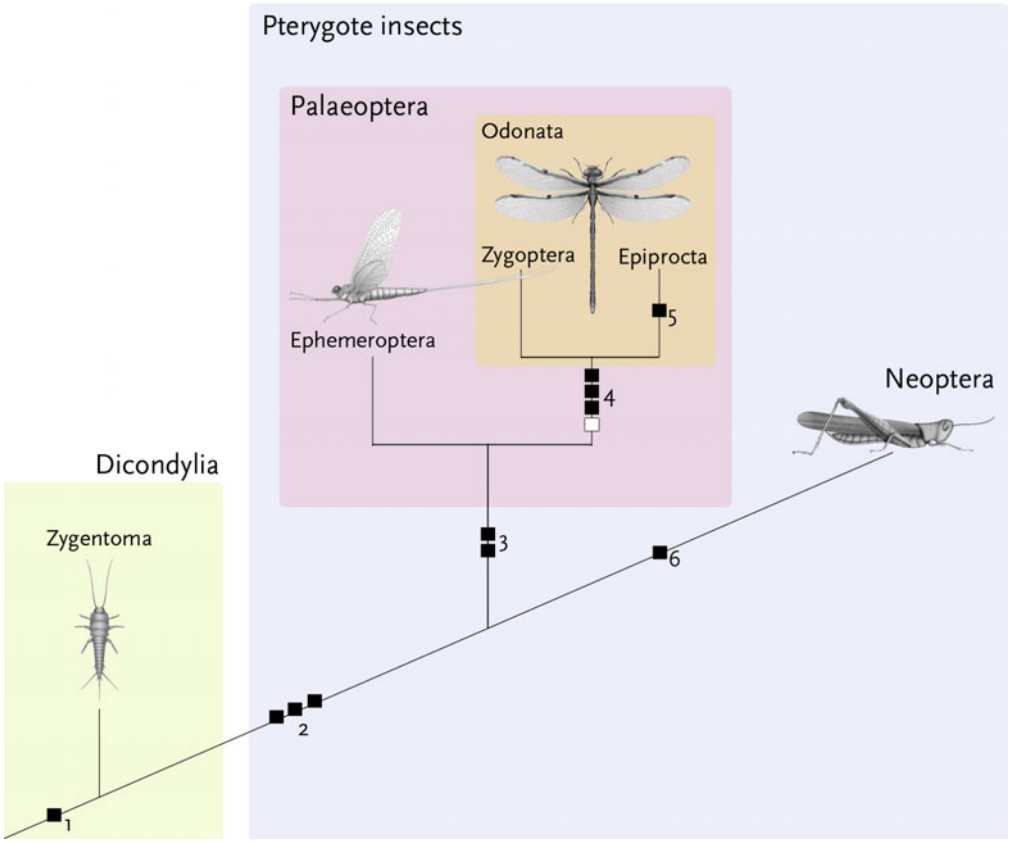

Figure 18 - Phylogeny of Insecta showing the Palaeoptera hypothesis (e.g. Blanke 2012a) including important character (apomorphies) groups 1-6 (character discussion Chapter 8.3.3). **1**: High amount of thorax musculature. **2**: Reduction of thorax musculature (Setup: Neoptera + Ephemeroptera + Odonata), wings on mesothorax and metathorax with indirect actuation and laterally positioned wing base sclerites (basalare). **3**: Reduction of thorax musculature and intermediate positioned wing base sclerites (basalare). **4**: Reduction of thorax musculature (Setup: Odonata), dorsal position of wing base sclerites (proximal costal plate = basalare), mostly direct wing actuation and seven thorax muscles (Ipcm9, Itpm7 through Itpm11 and IIscm8) unique for recent pterygote insects (plesiomorph). **5**: Reduction of IItpm2 (IIItpm2) **6**: Reduction of thorax muscles (Setup: Neoptera).

Ground pattern of Dicondylia – Conditions found in Zygentoma and Archaeognatha (Barlet 1953, 1954, 1967) make acceptable a high numbered thorax muscle setup for a stem-group representative of apterygote insects. (Character group 1)

Apomorphies of Pterygota – The wings and an indirect flight mechanism seem to be accompanied by a reduction in thorax musculature, e.g. on the one hand, the high number of thorax muscles observed in Zygentoma and Archaeognata, and on the other hand, the reduced number of thorax muscles when these are compared to Palaeoptera and Neoptera. Accordingly, a reduced muscle setup made up of the known muscles of Palaeoptera and Neoptera (cf. Chapter 8.3.2) for a stem-group representative of pterygote insects is most likely. This assumption is emphasized by Büsse & Hörnschemeyer (subm.), who mention an evolutionary trade-off relating to Muscle Ipcm9 of Odonata with counterparts in the mesothorax (IIpcm5) and metathorax (IIIpcm5) of Neoptera (cf. Friedrich & Beutel 2008). IIpcm5 and IIIpcm5 are, however, not present in Odonata. The pterygote ground pattern therefore has to show Ipcm9, IIpcm5 and IIIpcm5 as counterparts in the prothorax, mesothorax and metathorax. The lateral position of the wing base sclerite (basalare) is also related to a higher number of thorax muscles, because of the indirect flight mechanism's functionality (cf. Stem-species pattern of Odonata). It therefore represents a ground pattern character of pterygote insects. (Character group 2)

Apomorphies of Palaeoptera – The reduction of musculature is an apomorphy of Palaeoptera. It represents the first reduction within this group, because Ephemeroptera show fewer muscles than Neoptera but more than Odonata (e.g. Wilkommen 2008). The intermediate state of the wing base sclerites (basalare) mentioned in Ephemeroptera is the morphologically intermediate character state of the pterygote ground pattern and of Odonata and is therefore an apomorphy of Palaeoptera. (Character group 3)

Ground pattern of Odonata – The direct flight mechanism is related to a reduced muscle set up and dorsally positioned wing base sclerites (proximal costal plate = basalare) which both are apomorphies for Odonata. The muscles Ipcm9, Itpm7-tpm11 and IIscm8 are unique at least for a recent group of pterygote insects representing a plesiomorphy for Odonata. (Character group 4)

Apomorphies of Epiprocta – The reduction of thorax muscles IItpm2 (IIItpm2) are an apomorphy of Epiprocta [*Epiophlebia*+Anisoptera]. (Character group 5)

Apomorphies for Neoptera – The reduction of thorax musculature to the setup of Neoptera (cf. Friedrich and Beutel 2008). (Character group 6)

These ground pattern reconstructions represent a preliminary result, however. To complete the hypothetical muscle setup of the thorax of a pterygote ancestor all unique thorax muscles of Ephemeroptera had to be included and further investigation of adult Epiprocta and nymphal Zygoptera and *Epiophlebia* is preferable.

8.4. Concluding remarks

Due to their ability to fly, pterygote insects represent the most successful group of terrestrial arthropods. Insects were the first group of organisms in which flight evolved. The first insect fossils are found in the late Carboniferous (about 325 MYA) and the earliest winged insect fossils are known to date back to 420 and 390 MYA (cf. Grimaldi & Engel 2005). This time period is called Romer's Gap (Romer 1956). It is known for an exceptional lack of fossils in general, including insect fossils (e.g. Smithson 1985, Rolfe et al. 1994, Smithson et al. 1994, Clack 1998, Ruta et al 2003, Coates et al 2008). As a result, very little is known about when flight evolved (Wheat & Wahlberg 2013). We accordingly lack fossils for pterygote insects showing intermediate or even origin characters and forms from this important time period (e.g. Wheat & Wahlberg 2013) such as those presented for example in Clack et al. (1998) for fossil tetrapods.

The ground pattern of a stem-lineage representative provides important insights into the evolution and phylogeny of a group for example insects (e.g. Hennig 1969, 1981, 1982, Kristensen 1981, 1999, Clack 1998, Willmann 2003). It is indispensable to study the flight apparatus and flight of all major groups of Insecta, thus Neoptera (e.g. Snodgrass 1935, Nachtigall 1966, 1967, 2003, Nelson & Hanson 1968, 1971, Matsuda 1970, Zwick 1973, Brodsky 1979, 1986, Wootton 1979, Hörnschemeyer 1998, 2002, 2004, Nachtigall et al. 1998, Willkommen & Hörnschemeyer 2007, Friedrich & Beutel 2008, Willkommen 2008), Ephemeroptera (e.g. Dürken 1907, Knox 1935, Grandi 1947, Matsuda 1956, Brodsky 1970, 1971,1974, Klug 1994, Willkommen & Hörnschemeyer 2007, Willkommen 2008) and Odonata (Maloeuf 1935, Chao 1953, Asahina 1954, Tannert 1958, Russenberger 1960, Hatch 1966, Pfau 1986, Willkommen & Hörnschemeyer 2007, Willkommen 2008, Ninomiya & Yoshizawa 2009, Büsse et al. 2013, Genet et al. submitted, Büsse & Hörnschemeyer submitted) to obtain insights into the morphology and to elucidate the hypothetical ground pattern of each group.

A stem-group representative of pterygote insects is particularly vital in evaluating character states and for establishing a foundation and phylogenetic orientation for evolution.

In insect phylogeny, molecular studies are numerous. For example Wheeler et al. (2001), Kjer (2004), Yoshizawa & Johnson (2005), Terry & Whiting (2005), Cameron et al. (2006), Kjer (2006), Simon et al. (2009), Ishiwata et al. (2011) and Letsch et al. (2012) are only the names of a few scholars dealing with insect phylogeny; the number of intra-ordinal studies within the insects is countless. Nevertheless, a consensus of the phylogenetic relationships within the insects is currently lacking. The main challenge of calculating a robust phylogenetic tree is likely the "ancient rapid radiation phenomenon" (Rokas & Carroll 2006, Whitfield & Rokas 2007, Whitfield & Kjer 2008, Letsch et al. 2012). In other words, the first modern Neoptera evolved in a geologically short time span in the Early Mesozoic (Grimaldi & Engel 2005), followed by a long period of intra-ordinal diversification. The phylogenetic signal of the genetic data is

therefore minimal (Letsch et al. 2012).

We are however, coincidentally undergoing "a renaissance of insect morphology," as Beutel & Friedrichs (2008) describe it. More importance is attributed to morphology and taxonomy and not just because of a lack of "systematic sensitivity" that has sometimes occurred and is reflected by molecular biologists' (Norén & Jondelius 1997, Israelsson 1997) hilarious failure, when gut contents rather than tissue was analysed – as mentioned for example in Gee (2003). The insect head, for instance, has repeatedly proven useful for phylogenetic analyses (e.g. Hörnschemeyer et al. 2006, Beutel et al. 2010, Wipfler et al. 2011, Blanke et al. 2012a). Furthermore, morphological studies allow valuable insights into the phylogeny of several groups of insects like in Odonata (e.g. Misof 2002, Rehn 2003, Blanke et al. 2013b).

The absence of a phylogenetic signal in genetic data and renewed importance of morphology, whether to support and root molecular trees or present robust trees including or excluding genetic data distinctly shows just how important filling gaps and reviewing morphological studies are.

The morphological studies presented here and the resulting homology hypothesis of the Odonata and Neoptera thorax therefore enables phylogenetic access to one of the most important character complexes in insect evolution. The generalized Odonata thorax will allow simplified work and access to such a complex structure for future studies and promote the expansion of knowledge. What is more, it will provide a decisive aid for reconstructing the ground pattern of a stem-group representative of pterygote insects and elucidate the early evolution of the winged insects.

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Summary

The aim of my dissertation was to study the morphology and evolution of the thorax of damselflies and dragonflies (Odonata). One focus was the morphology of the thorax musculature and the homology between Odonata and a generalized Neoptera thorax as well as ground pattern of Pterygota (all winged insects). Furthermore, wing base skeletal element morphology was studied to extend and underscore the recent homology hypotheses. Beyond that, I examined the morphology, genetics and biogeography, and relating phylogeny of a very rare and enigmatic group of Odonata, *Epiophlebia*.

Epiophlebia present a unique position within the Odonata. The group of *Epiophlebia* is closely related to all dragonflies but represents the only group of Odonata not belonging to dragonflies (Anisoptera) or damselflies (Zygoptera). The four known species of *Epiophlebia* are adapted to an extreme habitat in Asian mountain regions. They prefer cold and swift-flowing mountain streams at an altitude ranging from 1000 to 3500 meters above sea level (stenoecious lifestyle). The habitats of the *Epiophlebia* species are highly separated from each other on the Asian continent. Their respective range shows no overlap areas today, which typifies speciation via spatial isolation (separation). Results of genetic investigation of three of the four species' DNA segments (sequences) show surprising, extreme homogeneity. These results lead to a biogeographical scenario, which assumes a shared habitat of *Epiophlebia* during the Würm ice age (approximately 20,000 years ago). When the warming phase started, *Epiophlebia*-populations were separated into distinct populations each located in a different glacial refuge (simplified, cold withdraw areas). This short time frame could explain the genetic homogeneity observed. Nevertheless, the question of the species status of *Epiophlebia* remains: Is there only one species – *Epiophlebia superstes* – in four different populations or are there four different species? During a subsequent morphological study the species status at least of *Epiophlebia laidlawi* Tillard, 1921 could also be confirmed.

Another study that draws directly on the genetic investigation of *Epiophlebia*, comprises a genetic sequence (S4-region of the 28s rRNA gene), which is suitable as a universal species identification tool for insects. Most insect specimens from all insect groups were successfully identified to species level with this tool. The investigation comprised 85 samples of 65 insect species, with at least one species per major clade of which the former represented a genus. We were able to demonstrate that our analysis system – which provides universal applicability and extended functionality – has advantages over the existing one (e.g. COI). The S4-method is applicable for degraded DNA that has, for example, been caused by aging, weathering or chemical influences.

Investigation of the Odonata thorax comprised three studies. Two of the musculature and sclerites of adult Zygoptera flight apparatus and one of the entire nymphal Anisoptera thorax musculature. The aim was to understand and highlight peculiarities of the odonatan thorax.

To obtain the data and reach the best overall result possible, traditional morphological methods – such as dissecting and hand drawing – were combined with one of the latest morphological methods, which included computer tomography (SRμCT) aided by 3D reconstruction.

By doing this, we discovered a total of 11 new, previously unknown muscles for Odonata. These morphological data were used to present the first complete homologization scheme of Odonata and neopterous insect thorax musculature. Furthermore, the homologies of the skeletal elements of the flight apparatus were confirmed and distinctly enhanced. This study also mark the first time muscle attachment points were discussed as important homology criteria. As a whole, these homology assessments allow unprecedented direct comparison between Odonata, which have a highly derived flight apparatus, and all other insects. Insights into the evolution and ground pattern of Odonata, even of all winged insects (Pterygota), were consequently gained. The homologies enable comparison and provide a complete new set of characters for subsequent analysis of the relationship (phylogenetic analysis) of Pterygota. A key, wing base sclerites' characteristic – the subalare – , points to the phylogenetic hypothesis of Paleoptera [Odonata+Ephemeroptera (mayflies)].

A generalized Odonata thorax that includes all recently known muscles will allow simplified work and access to the complex structure for future studies and will aid in furthering knowledge. This generalized thorax might be the initial point for a hypothetical ground pattern of pterygote insects and will allow insights into the development and evolution of the insect flight apparatus.

Zusammenfassung

In meiner Dissertation befasse ich mich mit der Morphologie und der Evolution des Thorax der Libellen (Odonata). Das Hauptaugenmerk liegt auf der Morphologie der Muskulatur und auf der Homologisierung der Libellenmuskeln mit einem generalisierten Grundbauplan der neopteren Insekten (vereinfacht – alle anderen geflügelten Insekten). Zudem wurden die Skelettelemente der Odonata eingehend behandelt und die bestehenden Homologiehypothesen wurden erweitert und gestützt. Darüber hinaus habe ich mich mit der Morphologie, Genetik, Biogeografie und der damit zusammenhängenden Verwandtschaft sehr seltener asiatischer Libellen (*Epiophlebia*) auseinander gesetzt.

Letztere Gruppe von asiatischen Libellen nimmt eine Sonderstellung innerhalb der Odonata ein. Die Gruppe *Epiophlebia* ist in einem gut begründeten Schwestergruppenverhältnis mit den Großlibellen positioniert (Epiprocta) und stellt somit die einzige Libellengruppe dar, die nicht zu den Groß- oder Kleinlibellen gruppiert werden kann. Die vier bekannten Arten von *Epiophlebia* sind an einen extremen Lebensraum angepasst. Sie bevorzugen kalte, schnell fließende Gebirgsbäche in Höhen von 1000-3500 Meter ü. NHN (Stenökie), sind aber auch an einen solchen Lebensraum gebunden. Die vier Arten kommen räumlich weit voneinander getrennt (disjunkte Verbreitung) auf dem asiatischen Kontinent vor. Ihre jeweiligen Verbreitungsgebiete haben heutzutage keinen Überlappungsbereich, was für eine Artaufspaltung durch räumliche Isolation (Separation) spricht. Daher sind die genetischen Ergebnisse von drei der vier Arten, die eine hohe Homogenität der einzelnen DNA Abschnitte (Sequenzen) aufzeigen, sehr überraschend. Diese Ergebnisse führen zu der Annahme eines biogeographischen Szenarios, welches einen gemeinsamen Lebensraum von *Epiophlebia* in der Würm-Eiszeit (vor ungefähr 20.000 Jahren) annimmt. Bei Rückgang der Eismassen wurde die *Epiophlebia*-Population durch ihre starke Stenökie in getrennte Populationen in Glazialrefugien – vereinfacht gesprochen kalt gebliebene Rückzugsgebiete – zurück gedrängt, in denen sie heute noch vorkommt. Dieser für evolutive Prozesse kurze Zeitraum kann die genetische Homogenität erklären. Dennoch bleibt die Frage nach dem Artstatus der vier *Epiophlebia*-Arten offen: Sind sie eine einzige Art? Dies würde bedeuten, dass sie eine voneinander räumlich getrennte Population darstellen. In diesem Fall wäre die Art *Epiophlebia superstes* Sélys, 1889. Oder sind sie wirklich vier getrennte Arten, so wie es der derzeitige Stand der Forschung annimmt. Die Frage nach dem Artstatus konnte zumindest für eine zweite Art *Epiophlebia laidlawi* Tillard, 1921 in einer darauf folgenden morphologischen Studie positiv beantwortet werden, so dass zwei Arten angenommen werden können.

Eine weitere Studie, die sich aus der genetischen Untersuchung von *Epiophlebia* ergeben hat, umfasst eine genetische Sequenz (S4-Region des 28s rRNA Gens), die für ein universelles Verfahren zur Art-Identifikation bei Insekten geeignet ist. Hierbei wurden die meisten aller Insektengruppen erfolgreich auf die Art identifiziert. Unsere Untersuchung umfasst 85 Proben aus 65 Insektenarten – mindestens eine Art aus jeder Großgruppe, die früher als Gattungen geführte wurde. Bei diesem sogenannten Barcoding, also dem Identifizieren von Arten mit Hilfe einer genetischen Analyse, kommt es häufig zu Schwierigkeiten. Wir

haben gezeigt, dass unser System große Vorteile gegenüber bereits bestehenden Systemen (z.B. COI) hat. Sie liegen vor allem in der universellen Anwendbarkeit sowie der hohen Funktionalität, da dieses Analyseverfahren auch bei stark degradierter DNA (z.B. durch Alterung, Verwitterung oder chemische Beeinflussung verursacht) anwendbar ist.

Die Untersuchungen zum Libellenthorax umfassen zwei Studien über adulte Kleinlibellen (Zygoptera). Hier wurden in einer Studie sowohl die Skelettelemente als auch die Muskulatur des Flugapparates untersucht. Eine weitere Studie umfasste die gesamte Muskulatur des Thorax bei Großlibellennymphen (Anisoptera-). Ziel war es, den wenig untersuchten Thorax der Zygoptera und der Libellen-Nymphen grundsätzlich besser zu verstehen und deren morphologische Eigenheiten aufzuzeigen, um diese Daten zu nutzen und um sie nach homologen Merkmalen zu untersuchen. Für die Analyse wurden traditionelle morphologische Verfahren, welches das Sezieren der Tiere und darauffolgendes Zeichnen beinhalten, mit modernen röntgentomographischen Verfahren (SRμCT), inklusive 3D-Rekonstruktion, kombiniert, um ein bestmögliches Ergebnis zu erhalten. Hierbei wurden insgesamt elf für Libellen bisher unbekannte Muskeln beschrieben.

Mit Hilfe dieser Daten wird das erste vollständige Homologie-Schema zwischen der Thoraxmuskulatur von Odonata und neopteren Insekten aufgestellt. Weiterhin werden die bereits aufgestellten Homologien der skelettalen Elemente des Flugapparates belegt und deutlich erweitert. Hierfür wurden unter anderem die Muskelansatzstellen als weiteres wichtiges Homologiekriterium erstmalig diskutiert. Die Gesamtheit dieser Homologiefeststellungen ermöglicht zum ersten Mal den direkten Vergleich von Libellen, die einen stark abgeleiteten Flugapparat besitzen, mit allen anderen geflügelten Insekten (Pterygota) vorzunehmen. Somit ist es möglich, Rückschlüsse auf die Evolution und deren Grundmuster von Libellen einerseits aber auch den gesamten Pterygota andererseits zu ziehen. Diese Homologien eröffnen neue Vergleichsmöglichkeiten und ein komplett neues Set an Merkmalen für spätere Verwandtschaftsanalysen der Pterygota. So gibt uns die Ausbildung eines der wichtigsten Teile des Flügelgelenks, des Subalare, Hinweise auf die Verwandschaftshypothese der Palaeoptera [Libellen+Eintagsfliegen].

Darüber hinaus war es möglich, einen generalisierten Libellenthorax mit allen derzeit bekannten Muskeln zu erstellen, was die Arbeit und die Identifikation von Muskeln im Libellenthorax erheblich vereinfacht und den Zugang zu diesem komplexen Gebiet deutlich erleichtert. Dieser generalisierte Libellenthorax ist der Ausgangspunkt für ein hypothetisches Grundmuster der Pterygota und kann tiefe Einblicke in die Entstehung und Evolution des Flugapparates der Insekten ermöglichen.

Erklärung

Hiermit erkläre ich, Sebastian Büsse, dass ich die vorliegende Arbeit ohne die unzulässige Hilfe Dritter und ohne die Verwendung anderer als der angegebenen Hilfsmittel angefertigt habe.

Diese Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Göttingen, den 15. Juli 2013

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Supplement

Table 1: Attachment Points Generalized Odonata Thorax

Abbrevation	Name	Origin	Insertion
Prothorax			
Dorsal longitudinal muscles			
Idlm1	Musculus prophragma-occipitalis	Apex of tergal apophysis 2	Median at the postocciput
Idlm3	M. prophragma-cervicalis	Tergal apophysis 1	Base of tergal apophysis 2
Idlm4	M. cervico-occipitalis dorsalis	Tergal apophysis 1	Median at postocciput
Dorsoventral muscles			
Idvm10	M. profurca-phragmalis	Apex of profurca	Apex of tergal apophysis 2
Idvm15	M. propleuro-coxalis superior	Anterolateral portion of tergite 1	Anterior procoxal rim
Idvm18	M. pronto-coxalis lateralis	Postero-lateral portion of tergite 1	Procoxal disk
Pleuro-coxal muscles			
Ipcm8	M. propleuro-trochanteralis	Episternum 1	Tendon of protrochanter
Ipcm9	M. protergro- trochanteralis	Lateral portion of tergite 1, close to the pleura.	Tendon of protrochanter
Sterno-coxal muscles			
Iscm2	M. profurca-coxalis posterior	External side of the base of profurca	Posterior procoxal rim
Iscm6	M. profurca-trochanteralis	External side of the base of profurca	Tendon of protrochanter
Sterno-pleural muscles			
Ispm1	M. profurca-apodemalis	Apex of profurca	Apodem of propleura

Tergo-pleural muscles			
ltpm3	M. pronoto-pleuralis anterior	Lateral side of tergite 1	Episternum 1
ltpm7	M. protergo-cervicalis posterior	Lateral part of tergite 1	Lateral of cervix membrane
ltpm8	M. protergo-cervicalis anterior	Most antero-lateral part of tergite 1	Lateral of cervix membrane
ltpm9	M. protergo-preepisternalis	Tergite 1, lateral of tergal apophysis 2	Base of preepisternalapodem 1
ltpm10	M. prosterna-coxalis dextra	Apex of right preepisternalapodem 1	Anterior of left procoxal rim
ltpm11	M. prosterna-coxalis sinister	Apex of left preepisternalapodem 1	Anterior of right procoxal rim
Ventral longitudinal muscles			
lvlm3	M. profurca-tentorialis	Apex of profurca	Cranial tentorial bar
lvlm7	M. profurca-mesofurcalis	Furca-branch 2	Furca 1
Mesothorax			
Dorsal longitudinal muscles			
IIdlm1	M. prophragma-mesophragmalis	Tergal apophysis 3	Tergal aposphysis 4 or posterior edge of the postnotum 2
Dorsoventral muscles			
IIdvm1	M. mesonoto-sternalis	Base of Mesofurca or preepisternal apodem	Postero-lateral edge of mesowing bud or at the tergum close to the tergal bridge

IIdvm3	M. mesonoto-trochantinalis posterior	Base of Mesofurca or inner wall of prefurca	Antero-lateral edge of mesowing bud or mesoscutum
IIdvm4	M. mesonoto-coxalis anterior	Antero-lateral, postero-lateral or at the anterior part of the edge of mesocoxa	Antero-lateral edge of mesowing bud or lateral part of the semi-detached scutal plate 2
IIdvm5	M. mesonoto-coxalis posterior	Mesocoxaldisk	Antero-lateral edge of mesowing bud or proximal edge of axillary plate 2
IIdvm6	M. mesocoxa-subalaris	Lateral part of tergite 2	Postero-lateral apodem of mesocoxa
Pleuro-coxal muscles			
IIpcm1	M. mesanepisterno-trochantinalis	Preepisternum 2	Lateral of the tergal apophysis 2 at tergite 2 or at the anterior edge of proximal costal plate 2
IIpcm2	M. mesobasalare-trochantinalis	Base of preepisternal apodem 2	Lateral of the tergal apophysis 2 at tergite 2 or at the cranial edge of proximal costal plate 2
IIpcm4	M. mesanepisterno-coxalis posterior	Interpleuralridge 2	Antero-external part of mesocoxa
IIpcm6	M. mesopleura-trochanteralis	Dorsal part of Katepisternum 2 or pleural bar between mesepisternum 2 and katepisternum 2	Tendon of mesotrochanter or lateral side of the mesocoxa
Sterno-coxal muscles			
IIscm1	M. mesofurca-coxalis anterior	Lateral base of Mesofurca	Antero-external ridge of mesocoxa

IIscm2	M. mesofurca-coxalis posterior	Lowermost part of mesofurca	Postero-lateral apodem of mesocoxa
IIscm3	M. mesofurca-coxalis medialis	Lateral base of mesofurca	Postero-lateral apodem of mesocoxa or ventral side of the furca branch 2
IIscm6	M. mesofurca-trochanteralis	Latero-external side of mesofurca or proximal side of the prefurca 2	Tendon of mesotrochanter
IIscm7	M. mesospina-metacoxalis	Preepisternal apodem	Anterolateral edge of metacoxa
Sterno-pleural muscles			
IIspm2	M. mesofurca-pleuralis	Apex of mesofurca	Interpleural ridge 2
Tergo-pleural muscles			
IItpm2	M. mesopleura-praealaris	Dorsal region of pleuralbar 2	Median semi-detached scutal plate 2
IItpm3	M. mesonoto-basalaris	Dorsal side of mesowing bud	Ventral side of mesowing bud
IItpm4	M. mesonoto-pleuralis anterior	Dorsal side of mesowing bud or Pleural bar 2	Ventral side of mesowing bud or Median semi-detached scutal plate 2
IItpm6	M. mesonoto-pleuralis posterior	Upper portion of interpleural ridge 2 or Pleural bar between mesepisternum and mesepimeron	Antero-dorsal edge of mesowing bud or lateral on the mesoscutellum

IItpm7	M. mesanepisterno-axillaris	Ventral part of epimeron 2	Lateral edge of mesowing bud or postregion of axillary plate 2
IItpm8	M. mesepimero-axillaris secundus	Ventral part of epimeron 2 or pleural bar between mesepimeron and katepisternum 2	Lateral edge of mesowing bud or at the epifulcrum of axillary plate 2
IItpm9	M. mesepimero-axillaris tertius	Dorsal part of epimeron 2 or posterior pleural process	Inner side of ventral portion of mesowing bud or axillary plate 2
IItpm10	M. mesepimero-subalaris	Interpleural ridge 2	Lateral edge of mesowing bud or posterior region of axillary plate 2
Ventral longitudinal muscles			
IIvlm6	M. mesospina-abdominosternalis	Posterior part of preepisternalapodem 3	Antecostal apodem
IIvlm7	M. mesofurca-abdominosternalis	Mesofurca	Within the Abdomen
Metathorax			
Dorsal longitudinal muscles			
IIIdlm1	M. mesophragma-metaphragmalis	Tergal apophys 4	Transversal ridge between abdomen and thorax or antecosta between abdomen and thorax
IIIdlm2	M. metanoto-phragmalis	Tergal apophysis 4 or scutellum	Transversal ridge between abdomen and thorax or proximal end of the tergal apophysis 4
Dorsoventral muscles			

IIIdvm1	M. mesonoto-sternalis	Base of metafurca	Postero-lateral edge of metawing bud or lateral region of the metascutum
IIIdvm3	M. metanoto-trochantinalis	Base of metafurca or cranial at the base of the metacoxa	Antero-lateral edge of metawing bud or postero-median region of the metascutum
IIIdvm4	M. metanoto-coxalis anterior	Anterio-lateral edge of metaocoxa or metacoxal disk	Antero-lateral edge of metawing bud or caudal of the semi-detached scutal plate 3
IIIdvm5	M. metanoto-coxalis posterior	Metacoxaldisk	Antero-lateral edge of metawing bud or axillary plate 3
IIdvm6	M. metacoxa-subalaris	Lateral part of tergite 3	Postero-lateral part of metacoxa
IIIdvm8	M. metanoto-phragmalis	Dorsal portion of the posterior ridge of epimeron 3 or posterior edge of the 1. abdominal tergite	Posterior end of metafurca or anterior edge of the furca invagination
Pleuro-coxal muscles			
IIIpcm1	M. metanepisterno-trochantinalis	Preepisternum 3 or intepleural ridge	Lateral of the tergal apophysis 3 at tergite 3 or proximal coxal plate 3
IIIpcm2	M. metabasalare-trochantinalis	Base of Preepisternal apodem 3	Lateral of the tergal apophysis 3 at tergite 3 or at the edge of the proximal coxal plate 3
IIIpcm4	M. metanepisterno-coxalis posterior	Interpleuralridge 3	Antero-external part or lateral of the posterior edge of metacoxa of the

IIIpcm6	M. mesopleura-trochanteralis	Dorsal part of Katepisternum 3 or Interpleuralridge 3	Tendon of metatrochanter or antero-lateral of the metacoxa
Sterno-coxal muscles			
IIIscm1	M. metafurca-coxalis anterior	Lateral base of Metafurca	Antero-external ridge of metacoxa
IIIscm2	M. metafurca-coxalis posterior	Lowermost part of metafurca	Postero-lateral apodem of metacoxa
IIIscm3	M. metafurca-coxalis medialis	Lateral base of metafurca	Postero-lateral apodem of metacoxa or antero-lateral edge of the metacoxa
IIIscm4	M. metafurca-coxalis lateralis	Apex of metafurca	Lateral base of metacoxa, at the border of pleurite
IIIscm6	M. metafurca-trochanteralis	Latero-external side of metafurca or proximal at the furca branch 3	Tendon of metatrochanter
Sterno-pleural muscles			
IIIspm2	M. metafurca-pleuralis	Apex of metafurca	Interpleural ridge 3
Tergo-pleural muscles			
IIItpm2	M. metapleura-praealaris	Dorsal region of the pleural bar between episternum 3 and epimeron 3	Median semi-detached scutal plate

IIItpm3	M. metanoto-basalaris	Dorsal side of metawing bud	Ventral side of metawing bud
IIItpm4	M. metanoto-pleuralis anterior	Dorsal side of metawing bud or pleural bar 3	Ventral side of metawing bud or median semi-detached scutal plate
IIItpm6	M. metanoto-pleuralis posterior	Upper portion of interpleural ridge 3	Antero-dorsal edgel of metawing bud proximal edge of axillary plate 3
IIItpm7	M. metanepisterno-axillaris	Ventral part of epimeron 3 or interpleural bar 3	Lateral edge of metawing bud or posterior region of the axillary plate 3
IIItpm8	M. metapimero-axillaris secundus	Ventral part of epimeron 3 or interpleural bar 3	Lateral edge of metawing bud or at the epifulcrum of the axillary plate 3
IIItpm9	M. metapimero-axillaris tertius	Dorsal part of epimeron 3 or posterior pleural process	Inner side of ventral portion of metawing bud or ventral part of axillary plate 3
IIItpm10	M. metapimero-subalaris	Interpleural ridge 3	Lateral edge of metawing bud or posterior region of the axillary plate 3
Ventral longitudinal muscles			
IIIvlm2	M. mesofurca-abdominosternalis	Proximal part of Metafurca (close to the prefurca invagination)	Within the abdomen (second abdominal sternite)
IIIvlm3	M. metaspina-abdominosternalis	Poststernum 3	the abdomen (second abdominal sternite)

Table 2: Comparatative Table Thorax Musculature

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

Friedrich & Beutel (2008)	Generalized Odonata thorax	Asahina (1954)	Willkommen (2008)	Wittig (1955)	Matsuda (1970)
Prothorax					
Idlm1	x	3	?	I dlm 10	op-t 3
Idlm2	-	-	?	0 dlm 1	op-t 2
Idlm3	x	2	?	I dlm 11b	cv(d)-t 1, t 14
Idlm4	x	1	?	0 dlm 2	op-t 1
Idlm5	-	-	?	I dlm 12	t 12
Idlm6	-	-	?	I dlm 12?	t 13
Idvm1	-	-	?	0 lm 7	op-cv 1
Idvm2	-	-	?	0 lm 7	op-cv 2
Idvm3	-	-	?	0 lm 8	op-cv 3
Idvm4	-	-	?	0 lm 5	t-s(cv) 1?
Idvm5	-	-	?	-	t-cv 1
Idvm6	-	-	?	0 lm 6	t-cv 2
Idvm7	-	-	?	-	t-cv 3
Idvm8	-	-	?	-	t-s(cv) 9
Idvm9	-	-	?	-	op-s 2, p-s 3
Idvm10	x	-	?	I ism 22	t-s 1
Idvm11	-	-	?	I ism 24	t-s 8
Idvm12	-	-	?	-	t-s 2
Idvm13	-	-	?	I dvm 15	t-ti(cx) 2
Idvm14	-	-	?	I dvm 16	t-ti(cx) 3
Idvm15	x	13	?	I dvm 17?	t-ti(cx) 1
Idvm16	-	-	?	I dvm 19	t-cx 5
Idvm17	-	-	?	I dvm 20	t-cx 6, t-cx 7
Idvm18	x	14 & 15	?	I dvm 21	t-cx 8
Idvm19	-	-	?	I dvm 18	t-tr 1

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

ltpm1	-	-	?	-	op-p 2
ltpm2	-	-	?	0 lm 9	op-p 1, t-p 3
ltpm3	x	12	?	l tpm 25	-
ltpm4	-	-	?	l tpm 26	t-p 14?
ltpm5	-	-	?	l tpm 27?	t-p 15?
ltpm6	-	-	?	-	t-p 1, t-p 2
-	ltpm7	4	?	?	?
-	ltpm8	5	?	?	?
-	ltpm9	7	?	?	?
-	ltpm10	9	?	?	?
-	ltpm11	10	?	?	?
lspm1	x	-	?	l zm 34	p-s1
lspm2	-	-	?	-	p-s 2
lspm3	-	-	?	-	p-s 6
lspm4	-	-	?	-	p-s 7
lspm5	-	-	?	-	p-s 5
lspm6	-	-	?	-	p-s 4
lspm7	-	-	?	-	p-s 10
lpcm1	-	-	?	-	cv-cx 3
lpcm2	-	-	?	-	cv-cx 1, cv-cx 2
lpcm3	-	-	?	-	p-ti(cx) 1
lpcm4	-	-	?	-	p-cx 4
lpcm5	-	-	?	l cpm 28	p-cx 5
lpcm6	-	-	?	-	p-cx 6, p-cx 9
lpcm7	-	-	?	-	p-cx 7
lpcm8	x	18	?	l cpm 29	p-tr 1, p-tr 2
-	lpcm9	17	?	?	?
lvlm1	-	-	?	0 vlm 4	cv-s 1, cv-s 4?
lvlm2	-	-	?	-	op-cv(v) 4

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

Ivlm3	x	11	?	0 vlm 3	s 1, s 2
Ivlm4	-	-	?	I vlm 14	s 14, s16
Ivlm5	-	-	?	-	s 17
Ivlm6	-	-	?	-	s 15
Ivlm7	x	41	?	I vlm 13	s 13
Ivlm8	-	-	?	-	s 11
Ivlm9	-	-	?	-	s 12
Iscm1	-	-	?	I bm 30	s-cx 5
Iscm2	x	16	?	I bm 33	s-cx 3
Iscm3	-	-	?	I bm 32	s-cx 6
Iscm4	-	-	?	-	s-cx 2
Iscm5	-	-	?	-	s-cx 4
Iscm6	x	19	?	I bm 31	s-tr 1
Iscm7	-	-	?	-	s-cx 1, s-cx 7
Mesothorax					
IIdlm1	x	25	MT.m	II dlm 35	t 14
IIdlm2	-	-	S.LP?m	II dlm 36, II dlm 37	t12, t13
IIdlm3	-	-	S.Esm	-	t-p 5, t-p 6
IIdvm1	x	23'	S.CmA	II dvm 40	t-ti 1, t-ti 2
IIdvm2	-	-	S.CmA	II dvm 41	t-ti 3
IIdvm3	x	23	-	-	t-cx 5
IIdvm4	x	26	PSL.Cm, S.CmP	II dvm 43	t-cx 6, t-cx 7
IIdvm5	x	27	SA.Cm, SA.Fm	II dvm 43	t-cx 8
IIdvm6	x	-	S.Trm	II cpm 53	t-tr 1
IIdvm7	-	-	-	II dvm 42	t-s 1
IIdvm8	-	-	-	II ism 44	t-s 8, t-s 7 ?
IIdvm9	-	-	A?.Pm	-	t-p 3
IItpm1	-	-	BA.Pm	II tpm 46a	t-p 4, t-p 20

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIltpm2	x	-	-	II tpm 47	t-p 7, t-p 8, t-p 9
IIltpm3	x	-	SrA.Pm, Ax.Pml	II tpm 46b ?	t-p 10, t-p 11, t-p 18
IIltpm4	x	28	-	-	t-p 12
IIltpm5	-	-	-	-	t-p 15
IIltpm6	x	31	-	II tpm 49	t-p 13
IIltpm7	x	33	-	II tpm 48	-
IIltpm8	x	32	Ax.PmS	-	t-p 14
IIltpm9	x	29/30	-	-	t-p 16
IIltpm10	x	34	-	II ppm 56	t-p 19
IIltpm11	-	-	-	-	t-p 17
IIltpm12	-	-	-	-	p 1
IIltpm13	-	-	-	?	?
IIppm1	-	-	-	II im 65a	p 2
IIppm2	-	-	-	II pm 54a, b	p 3
IIspm1	-	-	-	II ppm 55	p-s 1
IIspm2	x	35	-	II zm 61a	p-s 2
IIspm3	-	-	-	-	p-s 6
IIspm4	-	-	-	-	p-s 7
IIspm5	-	-	-	-	p-s 3
IIspm6	-	-	-	-	p-s 9
IIspm7	-	-	-	-	p-s 5
IIspm8	-	-	-	-	p-ti(cx) 1
IIpcm1	x	21	-	-	p-ti(cx) 2, p-ti(cx) 3
IIpcm2	x	22	-	II cpm 51	p-cx 4, p-cx 6
IIpcm3	-	-	P.Cm	-	p-cx 5
IIpcm4	x	36	BA.Trm	II cpm 52	p-tr 2
IIpcm5	-	-	P.Trm	II cpm 50	p-tr 1
IIpcm6	x	39	Fm	-	s14, s16
IIvlm1	-	-	-	-	s 15

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIvlm2	-	-	iFm	-	s 13
IIvlm3	-	-	-	II vlm 38	s 11
IIvlm4	-	-	-	-	s 12
IIvlm5	-	-	-	II vlm 39	p-s 13
IIvlm6	x	68	-	-	p-s 10
IIvlm7	x	41	F.CmA	-	s-cx 5
IIscm1	x	-	-	II bm 57	s-cx 3
IIscm2	x	-	-	II bm 60	s-cx 6
IIscm3	x	38	F.CmP	II bm 59	s-cx 2
IIscm4	-	-	-	II zm 61b	s-cx 4
IIscm5	-	-	-	-	s-tr1
IIscm6	x	40	-	II bm 58	s-cx 1
IIscm7	x	-	-	-	s-cx 7
Metathorax					
IIIdlm1	x	45	MT.m	III dlm 35	t 14
IIIdlm2	x	45'	S.LP?m	III dlm 36, III dlm 37	t 12, t13
IIIdlm3	-	-	-	III dvm 40	?
IIIdvm1	-	46'	S.Esm	III dvm 41	t-p 5, t-p 6
IIIdvm2	-	-	S.CmA	-	t-ti 1, t-ti 2
IIIdvm3	x	46	S.CmA	III dvm 43	t-ti 3
IIIdvm4	x	48	-	III dvm 43	t-cx 5
IIIdvm5	x	49	PSL.Cm, S.CmP	III cpm 53	t-cx 6, t-cx 7
IIIdvm6	x	46	SA.Cm, SA.Fm	III dvm 42	t-cx 8
IIIdvm7	-	-	-	III ism 44	t-tr 1
IIIdvm8	x	67	-	III tpm 46a	t-s 1
IIItpm1	-	-	A?.Pm	III tpm 47	t-p 3
IIItpm2	x	-	BA.Pm	III tpm 46b	t-p 4, t-p 20
IIItpm3	x	-	-	-	t-p 7, t-p 8

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIItpm4	x	50	SrA.Pm, Ax.Pml	-	t-p 10, t-p 11, t-p 18
IIItpm5	-	-	-	III tpm 49	t-p 12
IIItpm6	x	53	-	III tpm 48	t-p 15
IIItpm7	x	55	-	-	t-p 13
IIItpm8	x	54	-	-	-
IIItpm9	x	51/52	Ax.PmS	III ppm 56	t-p 14
IIItpm10	x	56	-	-	t-p 16
IIItpm11	-	-	-	-	t-p 19
IIItpm12	-	-	-	-	t-p 17
IIItpm13	-	-	-	?	?
IIIppm1	-	-	-	III im 65a	p 1
IIIppm2	-	-	-	III ppm 54a, b	p 2
IIIspm1	-	-	-	III ppm 55	p 3
IIIspm2	x	57	-	III zm 61	p-s 1
IIIspm3	-	-	-	-	p-s 7
IIIspm4	-	-	-	-	p-s 9
IIIspm5	-	-	-		p-s 5
IIIspm6	-	-	-	?	?
IIIpcm1	x	43	-	-	p-ti(cx) 1
IIIpcm2	x	44	-	III cpm 51	p-ti(cx) 2, p-ti(cx) 3
IIIpcm3	-	-	-	-	p-cx 4, p-cx 6
IIIpcm4	x	58	P.Cm	III cpm 52	p-cx 5
IIIpcm5	-	-	BA.Trm	III cpm 50	p-tr 2
IIIpcm6	x	62	P.Trm	-	p-tr 1
IIIpcm7	-	-	-	-	p-cx 8
IIIvlm1	-	-	Fm	-	s 14, s16
IIIvlm2	x	65	-	III vlm 64	s 20
IIIvlm3	x	66	-	-	s 12
IIIscm1	x	-	F.CmA	III bm 57	s-cx 5

Tab. 2: Homologisation of thoracic muscle nomenclatures used by several authors

"-" absent / "?" uncertain or no information

IIIscm2	x	-	-	III bm 60	s-cx 3
IIIscm3	x	61	-	III bm 59	s-cx 6
IIIscm4	-	-	F.CmP	-	s-cx 2
IIIscm5	-	-	-	-	s-cx 4
IIIscm6	x	63	-	III cpm 58	s-tr 1

Digital Version of Dissertation: S Büsse 2013

Bestätigung der Übereinstimmung

Hiermit versichere ich, Sebastian Büsse, dass die digitale Version mit der schriftlichen wissenschaftlichen Abhandlung übereinstimmt.

Göttingen, den 15. Juli 2013

Curriculum **V**itae

Sebastian Büsse

Diplom Biologe

Persönliche Daten

Anschrift	Güterbahnhofstr. 2 37073 Göttingen
Geburtstag/-ort	29.03.1982 in Hildesheim
Staatsangehörigkeit	deutsch
Familienstand	ledig

Bildungsweg

Seit 10.2010	Georg–August–Universität Göttingen, Dissertation Biologie Titel: The Thorax of Odonata (Insecta) - including remarks on evolution and phylogeny Abschlussnote: magna cum laude. Offizielle Verkündung: 01.11.2013
10.2005 – 09.2010	Georg–August–Universität Göttingen, Diplom-Studiengang Biologie Akademischer Grad: Diplom-Biologe Abschlussnote: 1,1 Titel : Vergleichend morphologische Untersuchungen an prothorakalenBeinen von Tarsenspinnern(Insecta: Embioptera) verschiedener Ökotypen
10.2003 – 09.2005	Georg–August–Universität Göttingen, Diplom-Studiengang Chemie
08.2002 – 05.2003	Zivildienst in der Behindertenbetreuung, Diakonische Werke Himmelsthür in Hildesheim e.V.
08.1999 – 06.2002	Goethegymnasium Hildesheim Allgemeine Hochschulreife (Abitur)

Fähigkeiten

Sprachkenntnisse	Englisch (sehr sicher in Wort und Schrift), Spanisch (Grundkenntnisse)
Interessengebiete	(Natur-) Fotografie, Wandern, Volleyball, Gitarre spielen und Musik hören
Analyseverfahren	Erstellung und Umgang mit synchrotron radiation micro computed tomography (SRµCT) Datensätzen Serial block-face scanning Elektronenmikroskopie (SBFSEM) Rasterelektronenmikroskopie (REM und ESEM) Transmissionselektronenmikroskopie (TEM) Laserscanmikroskopie (CLSM) Lichtmikroskopie CE-Analyse, Sequenzierung, PCR Digital-Fotografie, Focus stacking
Computerprogramme	Amira (Programm für 3D-Computer Rekonstruktion) (umfassende Kenntnisse) Paup, Mr. Bayes (Phylogenetische Analyse Programme) (Anwenderkenntnisse) MEGA (DNA Analyse Programm) (Anwenderkenntnisse) Microsoft Office (Word, Excel, PowerPoint) (umfassende Kenntnisse) Adobe Photoshop, Adobe Lightroom, Adobe Illustrator, Adobe Indesign (umfassende Kenntnisse)

Sebastian Büsse

Diplom Biologe

Universitäre Tätigkeiten

Seit 05.2013	Eigenverantwortliche Betreuung einer Bachelorarbeit inklusive Vorbereitender Praktika zum Thema „The thorax musculature of Zygoptera (Insecta: Odonata) nymphs“
11.2012 – 04.2013	Organisations-Komitee der Tagung der Deutschen Gesellschaft für allgemeine und angewandte Entomologie (DGaaE) 2013 in Göttingen.
09.2012 – 06.2013	Eigenverantwortliche Betreuung einer Diplomarbeit zum Thema „Ontogenetic development in *Epiophlebia* (Insecta: Odonata)“
05.2012 – 03.2013	Koordination und Organisation der Forschungsprojekte in Bolivien der Abteilung Morphologie, Systematik und Evolutionsbiologie
07.2011 – 08.2011	Eigenverantwortliche Betreuung eines Laborpraktikums (3 Wochen Block) zum Thema „Der Thorax von neopteren Insekten mit Methoden Vertiefung“
04.2011 – 07.2013	Eigenverantwortliche Vorlesung und Praktikum, Teil „Porifera und Cnidaria“ im Rahmen des Kurses „Morphologie und Systematik für Anfänger“
02.2011 – 05.2013	Koordination der studentischen Hilfskräfte für Forschung und Lehre der Abteilung Morphologie, Systematik und Evolutionsbiologie
04.2009 – 06. 2009	Eigenverantwortliche methodische Betreuung (Semi-Dünnschnitte, REM & TEM) einer Bachelor Absolventin
04.2007 – 09.2009	Tutor in den Kursen: „Morphologie und Systematik für Anfänger“, „Morphologie und Systematik I: Grundkurs für Fortgeschrittene“, „Morphologie und Systematik III: Elektronenmikroskopie (TEM & REM) “,
08.2008 – 03.2009	Hilfswissenschaftler in der Abteilung Entwicklungsbiologie, Georg-August-Universität Göttingen
02.2007 – 09.2010	Hilfswissenschaftler in der Abteilung Morphologie, Systematik & Evolutionsbiologie, Georg-August-Universität Göttingen
02.2006 – 04.2006	Volontär auf der Iguana Research & Breeding Station Islas de la Bahia - Isla de Utila, Honduras

Reviewer-Tätigkeit

Seit 01.2013	PLoS one

Auszeichnungen

In 2012	ICO Award – Bester Vortrag
In 2011	Lopi-Preis – Bester studentischer Vortrag

Mitgliedschaften

Seit 11.2011	Gesellschaft deutschsprachiger Odonatologen (GdO e.v.)
Seit 03.2012	World Dragonfly Association (WDA)
Seit 03.2013	Gesellschaft für Biologische Systematik (GfBS) & Junge Systematiker (JuSys)

Sebastian Büsse

Diplom Biologe

Forschungs- & Sammelreisen

11.2011 – 12.2011 & 03.2012 – 04.2012	Bolivien
07.2012 – 09.2012	Japan

Kooperationen

Seit 02.2013	Coleorryncha Projekt: Christian Fischer, JFB Institute of Zoology and Anthropology, Department of Morphology, Systematic and Evolutionary Biology, Georg-August-University Göttingen, Germany.
Seit 12.2011	*Epiophlebia* Projekt: Kazunori Yoshizawa, Department of Systematic Entomology, Hokkaido University, Sapporo, Japan.
Seit 10.2012	DNA Projekte: Philipp von Grumpkow & Susanne Hummelm, JFB Institute of Zoology and Anthropology, Department of Historical Anthropology and Human ecology, Georg-August-University Göttingen, Germany.
Seit 04.2009	Embioptera Projekt: Janice Edgerly, Chair, Biology, Santa Clara University, Santa Clara, CA, USA.

Sammlungsarbeit

10.2012	University Museum of Zoology at University of Cambridge in Cambridge, UK
08.2012	Dragonfly Kingdom Nature Park in Shimanto City, Japan
03.2012	Museo de Historia Natural "ALCIDE d'ORBIGNY" de Cochabamba, Bolivien
11.2011	Museo de Historia Natural Noel Kempff in Santa Cruz, Bolivien
02.2011	Zoologisches Forschungsmuseum Alexander Koenig in Bonn, Deutschland

Erlangte Förderungen

2012	Kongress- und Vortragsreisenstipendium des Deutschen Akademischen Austausch Dienstes (DAAD) für den international Congress of Odonatology, Odawara, Japan.
2010	24 Stunden Strahlzeit am Swiss Light Source (SLS) in Villingen, Schweiz (Proposal no. 20100088, November 2010) für Hochauflösende Röntgentomographie von Insekten.
2010	48 Stunden Strahlzeit am Deutschen Elektronen Synchrotron (DESY) in Hamburg, Deutschland (Proposal no. I-20090257, April 2010) für Hochauflösende Röntgentomographie von Arthropoden.
2009	48 Stunden Strahlzeit am Deutschen Elektronen Synchrotron (DESY) in Hamburg, Deutschland (Proposal no. I-20090102, Aug. 2009) für Hochauflösende Röntgentomographie von Insekten.
2009	72 Stunden Strahlzeit am Helmholzzentrum Berlin (BESSY II) in Berlin, Deutschland (Proposal no. 2009_90372, Aug. 2009) für Hochauflösende Röntgentomographie von Insekten

Göttingen, den 25. Juli 2013

Sebastian Büsse
Diplom Biologe

Publikationen (Originalarbeiten, * zum Antrag eingereicht)

***Büsse S** & Hörnschemeyer T (submitted) The nymphal thorax musculature of Anisoptera (Insecta: Odonata) and its evolutionary relevance. BMC Evolutionary Biology.

Genet C, **Büsse S** & Hörnschemeyer T (submitted) The thorax morphology of Zygoptera (Insecta: Odonata) – the skeletal system. Arthropod Structure and Development.

von Grumbkow P, **Büsse S**, Hörnschemeyer T, Mazanec J, Tröster G & Hummel S (submitted) Analysis system for taxonomic identification of Insecta species applicable to strongly degraded DNA using the nuclear 28S-rRNA gene. Systematic & Diversity.

***Büsse S**, Genet C & Hörnschemeyer T (2013) Homologization of the Flight Musculature of Zygoptera (Insecta: Odonata) and Neoptera (Insecta). PLoS ONE 8(2): e55787. doi:10.1371/journal.pone.0055787

***Büsse S**, von Grumbkow P, Hummel S, Shah DN, Tachamo Shah RD, et al. (2012): Phylogeographic Analysis Elucidates the Influence of the Ice Ages on the Disjunct Distribution of Relict Dragonflies in Asia. PLoS ONE 7(5): e38132. doi:10.1371/journal.pone.0038132

Edgerly JS., **Büsse S** & Hörnschemeyer T (2012): Spinning behaviour and morphology of the spinning glands in male and female *Aposthonia ceylonica* (Enderlein, 1912) (Embioptera: Oligotomidae). Zoologischer Anzeiger 251(4):297-306. http://dx.doi.org/10.1016/j.jcz.2011.12.006

Veröffentlichte Abstracts

Hörnschemeyer T & **Büsse S** (2011): Ontogenetic development of *Epiophlebia* (Odonata: Anisozygoptera) – first results. BioSystematics 2011 Meeting, Berlin, Germany. ISBN 978-3-921800-68-3

Büsse S & Hörnschemeyer T (2011): Ontogenesis of *Pyrrhosoma nymphula* (Odonata: Zygoptera) – egg to first instar studied with SRµCT. BioSystematics 2011 Meeting, Berlin, Germany. ISBN 978-3-921800-68-3

Büsse S & Hörnschemeyer T (2011): Comparative morphological study of prothoracic legs of Embioptera (Insecta) from various ecotypes via SRµCT. BioSystematics 2011 Meeting, Berlin, Germany. ISBN 978-3-921800-68-3

Eingeladene Vorträge

Büsse S & Hörnschemeyer T (2013) The thorax musculature of Odonata and the homology with Neoptera. International Congress of Odonatology (ICO2013), Freisingen, Germany.

Büsse S, von Grumbkow P, Hummel S, Shah DS, Tachamo Shah RD, Li J, Zhang X, Yoshizawa K, Wedmann S & Hörnschemeyer T (2012): Only one species of *Epiophlebia*? – first DNA analysis of all Epiophlebia species (Insecta: Odonata). International Congress of Odonatology (ICO2012), Odawara City, Japan. **(ICO Award 2012 – Best Talk)**

Vorträge

Büsse S, von Grumbkow P & Hörnschemeyer T (2013): Phylogeographic Analysis of the Disjunct Distribution of Relict Dragonflies in Asia – *Epiophlebia* (Insecta: Odonata). Jahrestagung der Deutschen Geselschaft für allgemeine und angewandte Entomologie (DGaaE) 2013, Göttingen, Germany.

Büsse S (2013): Is *Epiophlebia laidlawi* Tillyard, 1921 (Insecta: Odonata) a good species? 32. Jahrestagung der Geselschaft deutschsprachiger Odonatologen (GdO) 2013, Fulda, Germany.

Büsse S, von Grumbkow P, Yoshizawa K, Wedmann S & Hörnschemeyer T (2012): Molecular and phylogeographic analysis of the relict dragonflies of *Epiophlebia* (Insecta: Odonata). XXIV International Congress of Entomology (ICE2012), Daegu, South Korea.

Büsse S, Blanke A, Misof B & Hörnschemeyer T (2012): The nymphal head of *Epiophlebia laidlawi* (Insecta: Odonata) – morphological study with focus on the musculature. 31.Jahrestagung der Geselschaft deutschsprachiger Odonatologen (GdO) 2012, Freiberg, Germany.

Fischer C, Helmker B & **Büsse S** (2011): The biological context and evolution of Pendergrast's organ of Acanthosomatidae (Heteroptera, Pentatomoidea). III. Congreso de Entomología, Santa Cruz, Bolivia.

Büsse S & Hörnschemeyer T (2011): Morphometrics of spinning glands in Embioptera (Insecta) of different ecotypes investigated with SRµCT. 2. International Congress on Invertebrate Morphology, Bosten, USA.

Hörnschemeyer T & **Büsse S** (2011): Investigation of the ontogenetic development of Odonata using synchrotron radiation micro-tomography (SRµCT). 2. International Congress on Invertebrate Morphology, Bosten, USA.

Büsse S & Hörnschemeyer T (2011): Phylogenetic position and larval morphology of *Epiophlebia laidlawi* (Odonata: Insecta) – first results. 30. Jahrestagung der Geselschaft deutschsprachiger Odonatologen (GdO) 2011, Lübeck, Germany.
(Lopi-Preis – Best Student Talk)

Büsse S & Hörnschemeyer T (2011): Comparative morphological study of prothoracic legs of Embioptera (Insecta) from v arious ecotypes via SRµCT. BioSystematics 2011 Meeting, Berlin, Germany.

Poster

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