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Bc. Kateřina Rosová

The growth and development of wings during ontogeny with emphasis on Palaeodictyoptera

Růst a vývoj hmyzích křídel v průběhu ontogeneze se zřetelem na skupinu Palaeodictyoptera

Diploma thesis

Supervisor:

Doc. RNDr. Jakub Prokop, Ph.D.

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Declaration:

I declare that I have written the thesis independently and all sources and literature used have been cited properly. The work has not been used as a thesis to obtain a different or similar type of university qualification.

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In Prague, 10th August, 2020

ABSTRAKT

Tato práce se zaměřuje na série ontogenetických stádií fosilních zástupců hmyzu z řádu Palaeodictyoptera pocházejících z pozdního karbonu Kuznětské pánve v Ruské federativní republice. Důraz byl kladen na vývoj křídel u druhů *Tchirkovaea guttata* a *Paimbia fenestrata* náležících do čeledi Tchirkovaeidae a jeho srovnání s vývojem křídel u recentního hmyzu. Materiál těchto fosilních druhů byl předchozími autory považován za důkaz unikátního vývoje křídla u paleozoického hmyzu. Předpokládalo se, že larvy tohoto hmyzu měly křídla, která byla artikulována a plně pohyblivá již během ranějších fází postembryonálního vývoje, a že tato postupně rostoucí křídla měnila s postupem vývoje svou polohu z podélné na kolmou k ose těla. Navíc měl vývoj zahrnovat dva či více subimaginálních instarů, což předpokládá vícenásobné svlékání plně okřídlených instarů. Po důkladném prostudování a následném porovnání fosilních důkazů s vývojem křídel u recentní jepice *Cloeon dipterum* bylo zjištěno, že údajná řada juvenilních, subimaginálních a imaginálních křídel druhů *T. guttata* a *P. fenestrata* neposkytuje jasný důkaz, který by podpořil původní hypotézu o vývoji křídla u paleozoického hmyzu, jak jej uváděli předchozí autoři. Výsledky naopak naznačují, že tyto fosilie křídel ve skutečnosti představují křídelní pochvy, které jsou dobře známé u recentních zástupců hemimetabolního hmyzu.

Klíčová slova:

ABSTRACT

The series of two fossil species belonging to the order Palaeodictyoptera from the Late Carboniferous of Kuznetsk Basin in Russia were re-examined. The two species as *Tchirkovaea guttata* and *Paimbia fenestrata* were investigated with emphasis on the wing growth and development in comparison with the structure of developing wings in recent mayflies. This fossil material of *T. guttata* and *P. fenestrata* was long considered by previous authors as undisputed evidence for a unique type of wing development in the Palaeozoic insects. The idea was that the larvae of these insects possessed the wings, which became articulated and fully movable already earlier during the postembryonic development and that these gradually growing wings changed their position from longitudinal to perpendicular to the body axis. Moreover, the development was supposed to include two or more subimaginal instars, implying that the fully winged instars moulted several times during postembryonic development. After detailed study of the available fossils and subsequent comparison of the fossil evidence with the development of wings in the recent mayfly *Cloeon dipterum* it was discovered, that the alleged series of immature, subimaginal and imaginal wings of *T. guttata* and *P. fenestrata* do not provide clear evidence that would support the original idea of wing development in the Palaeozoic insects as interpreted by the previous authors. On the contrary, the results of this thesis suggest, that the fossil specimens of the supposed wings in fact represent the wing pads, a developing wing inside the sheath well known in the recent hemimetabolous insects.

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1. Introduction

The superorder Palaeodictyopterida (comprises Palaeodictyoptera, Megasecoptera, Permothemistida and Diaphanopterodea) inhabited our planet from the Late Carboniferous to the end of Permian. The group is traditionally placed among the early diverged lineages of winged insects (Pterygota) and together with Odonata and Ephemeroptera forms the group Palaeoptera (Engel et al. 2013).

This group is known only from the fossil record, where one can usually find only the adult body parts or their fragments, scarcely can be discovered isolated larval wing pads or wing pad sheaths or the whole larval exuviae. Of very rare occurrence are the series of multiple larval wing pads belonging to different ontogenetic stages of the same or related species.

It appears hard to determine the exact systematic position or the type and course of ontogeny of these, since the wing pads possess only incomplete tracheation or venation. Whether the preserved fossils represent the developing wings themselves or the larval wing pads with developing wings inside (known in the extant hemimetabolous orders) is not always clear. In recent orders the wing pads are wholly and immovably fused with notum (except for Orthoptera and Odonata for their rotation) meanwhile in Palaeodictyopterida the larval wing pads were apparently medially articulated (Prokop et al. 2017a), which raises the question whether they could be at least partially movable.

In this thesis is summarised the existing knowledge concerning the ontogenetic development of wings in Palaeodictyopterida and revised the rare material consisting of the unique series of the larval wing pads assigned to *Tchirkovaea guttata* Zalessky, 1931 (Palaeodictyoptera: Tchirkovaeidae) and additional fossil specimens of *Paimbia fenestrata* Sinitshenkova, 1979 from the Late Carboniferous of Russia (Sinitshenkova 1979). This material is considered as evidence for the development of wings during the consequent larval stages together with other body structures. The obtained data will be compared with the information about the development of the wing pads and their tracheation and venation in the course of ontogeny in recent mayfly, *Cloeon dipterum* (Linnaeus, 1761). The main aim of this comparison is to outline possible conclusions on the growth of wings in the ontogeny and even on the course of postembryonic development in one of the oldest lineages of winged insects (Pterygota) (Brauckmann et al. 1996).

1.1. The postembryonic development of recent insects

It seems that the appearance of wings in insects is closely related to the subsequent emergence of hemimetaboly and the final moult from larva to adult, since moulting of the membranous wings in ametaboly would be complicated (Belles 2020). Reminiscence of this transition between ametaboly and hemimetaboly can be observed today in the metamorphosis in recent Ephemeroptera (Snodgrass 1954, Schaefer 1975), called prometaboly. The subimaginal instar has to go through a final moult with complicated ecdysis of the functional wings, resulting in selective pressure for such modifications in the development that prevent the difficulties to happen. The subimagos of some species do not shed the subimaginal wing cuticle and in other species they became neotenic, capable of reproduction without the need to moult into adult (Sehnal et al. 1996).

Since the next chapters are focused on the growth of the wings in the course of postembryonic development of Palaeozoic and modern insects, it is convenient to depict the types of metamorphosis (morphological change in the form of structure of body in the course of ontogenetic development) which are recognized in recent insect fauna and present the terminology used to describe developmental stages of insects with different types of metamorphosis.

The most popular classification of insect postembryonic development is the simple division into ametabolan, hemimetabolan and holometabolan insects, but more detailed classification was already introduced by Berlese (1913) and it is one of the commonly used today (Belles 2020).

Berlese (1913) divided the metamorphosis of living insects into four main categories: ametaboly, hemimetaboly, neometaboly and holometaboly. The author distinguished "ametabolia genuina" or actual ametaboly, occurring in primarily apterygote insects, and "ametabolia acquisita" or acquired ametaboly, representing one of the forms of hemimetaboly. Hemimetaboly was thus subdivided based on the rate of similarity between immatures and their adults into pseudoametaboly in all hemimetabolous species with apterous adults, paurometaboly in majority of terrestrial hemimetabolous species with winged adults, and heterometaboly in amphibious Neuroptera and in species of Homoptera with subterranean immatures. Berlese assumed that the neometaboly present in some Homoptera was transitional type of metamorphosis between hemimetaboly and holometaboly, characterized by having completely immobile immature stages. Holometaboly was then divided into the typical holometaboly and hypermetamorphosis, comprising also polymetamorphosis.

Weber (1949) designed a much more complex classification. He called ametaboly as epimataboly and proposed the special type of development for Ephemeroptera, so called prometaboly, based on the presence of subimaginal instar. Odonata and Plecoptera with their relatively gradual postembryonal development of adult features and aquatic immatures were classified as archimetabola. The neometabola first proposed by Berlese were divided by Weber into four categories: homometaboly in Phylloxeroidea (Sternorrhyncha) with external wing primordia present only in the last immature stage; parametaboly in the males of Coccomorpha (Sternorrhyncha) where after feeding wingless immature instars follow one or two nonfeeding stages with external wing primordia; allometaboly in Aleyrodoidea (Sternorrhyncha) where all juvenile stages are wingless and the last stage immobile; and finally, remetaboly in Thysanoptera with wingless instars followed by one mobile and one to three immobile stages with wing primordia.

In holometaboly Weber differentiated between euholometaboly, the most common type, and aoholometaboly, present in Megaloptera with aquatic juveniles similar to the adults and fully motile pupae. He also distinguished three types of hypermetamorphosis, polymetaboly in parasitic Coleoptera and Strepsiptera, hypermetaboly in Meloidae (Coleoptera) and cryptometaboly for viviparous Phoridae (Diptera).

This thesis will follow the most widely used classification in many entomology textbooks and publications (e.g., Sehnal et al. 1996; Beutel et al. 2014; Gullan & Cranston 2014; Belles 2020) dividing insect metamorphosis into three main types: ametaboly, hemimetaboly and holometaboly (Fig. 1). The insect orders that do not possess wings (Archaeognatha and Zygentoma) are all considered ametabolan, the insects with wings (Pterygota) consist of Endopterygota with the pupal stage that undergo holometaboly, and the rest of the insect orders with hemimetaboly. The traditional classifications of Berlese and Weber were created with little or no phylogenetic sense (Belles 2020), but they are useful in discussions of the more specialised modifications of insect development within the main categories of the types of insect metamorphosis (e.g. Sehnal et al. 1996; Štys & Šobotník 1999; Rédei & Štys 2016) and will be used in this sense.
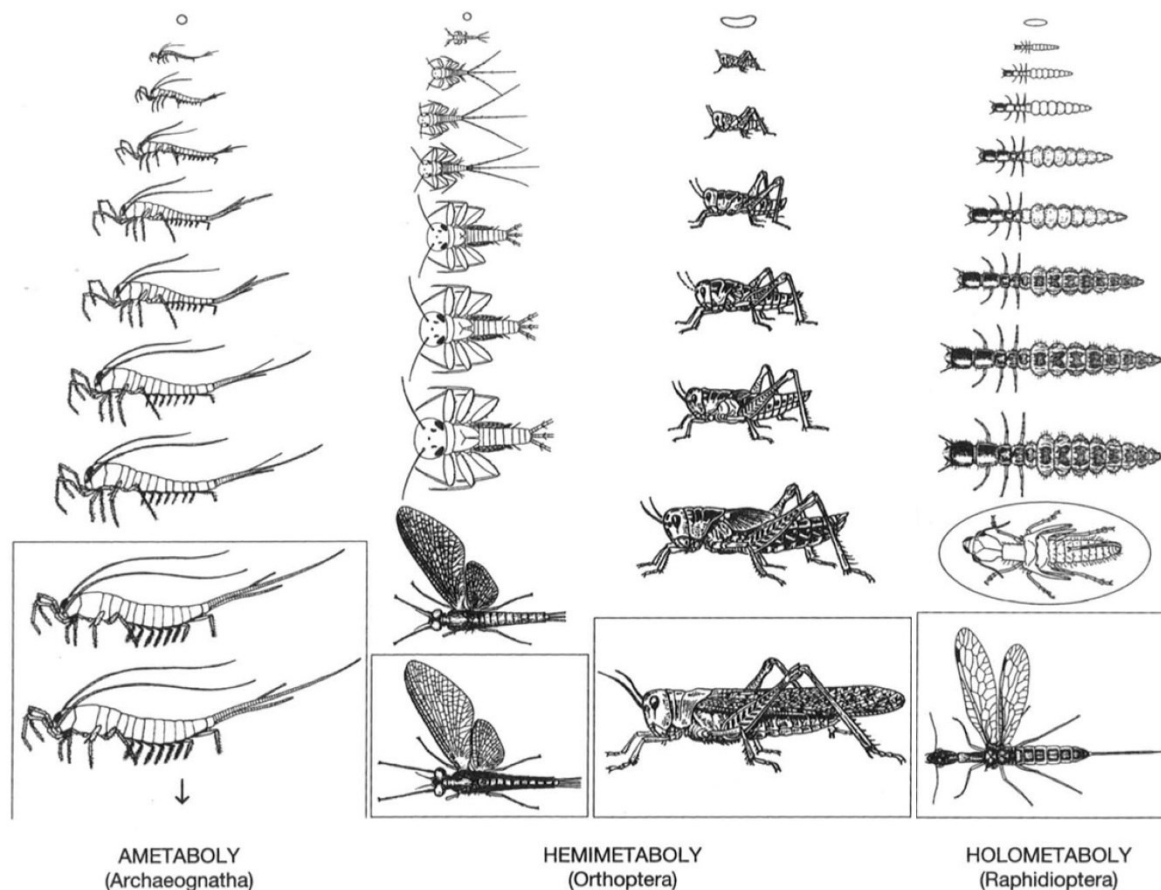
Figure 1: Three basic types of development of modern insects (Sehnal et al. 1996, modified).

If the insects grow gradually without any abrupt morphological change between immature and adult (except the presence of functional genitalia) and continue to moult after reaching sexual maturity, they are termed ametabolous (Beutel et al. 2014; Gullan & Cranston 2014). Ametaboly can be found in basal hexapods (Collembola, Protura and Diplura) and in apterygote insect lineages as Archaeognatha and Zygentoma (Sehnal et al 1996).

Hemimetaboly is a relatively gradual development, which leads to the emergence of sexually mature adults that no longer moult. The compound eyes are already present in immature stages and the adult structures, such as the wings and genitalia, develop gradually, but their main growth occurs in the later immature instars and is most apparent during the last moult into adult (Chapman et al. 2013; Truman & Riddiford 2019). This type of development is often termed incomplete metamorphosis in contrast to holometaboly, which is referred to as complete metamorphosis (Sehnal et al. 1996). Hemimetabolous insects usually have only one adult stage with fully developed wings, except for Ephemeroptera with one winged subimaginal instar that precedes the adult stage (Chapman et al. 2013). Hemimetaboly is present in three insect groups: Palaeoptera, Polyneoptera and Paraneoptera (Belles 2020).

Holometaboly represents an autapomorphy for all holometabolous insects forming a monophyletic group Holometabola or Endopterygota. In contrast to previous types of postembryonic development, immatures of Holometabola differ greatly from their adults in morphological characteristics and also in lifestyle. Some imaginal structures such as wing pads and genitalia develop concealed below the immature cuticle and become external for the first-time during metamorphosis in the main transitional phase between immature and adult – the pupa. Other adult structures, such as compound eyes, appear in this stage for the first time ever (Beutel et al. 2014; Belles 2020).

1.2. The immature stages of insects

The immature stages of insects have been termed variously throughout the course of entomological research. According to Comstock (1918) the term larva was for the first time applied to the juvenile stages of all insects, no matter what type of development they undergo. The author claimed that in the later nineteenth century the term larva became restricted to the immatures of Holometabola and the term nymph, also used for the term pupa, was used to distinguish the juveniles of hemimetabolous insects. Comstock (1918) also introduced the use of the term naiad to entomology to refer to the aquatic immatures of Ephemeroptera, Odonata and Plecoptera, which differ from their adults to a bigger extent than in other insects with incomplete metamorphosis.

Today the terms larva, nymph and naiad are used variably by different authors without consensus. According to Bybee et al. (2015) "*these terms should be used to define general habitat and postembryonic developmental similarities, ...*", requesting "*a strict and categorical use*". Following the Berlese (1913) classification of the types of insect metamorphosis the authors suggest, that the term larva should be used solely for immatures of insects with complete metamorphosis, nymph for insects with paurometaboly (gradual metamorphosis) and naiad for hemimetabolous Ephemeroptera, Odonata and Plecoptera with incomplete metamorphosis and juveniles differing greatly from the adults in morphology and lifestyle.

Muzón et al. (2016) disapproved the approach chosen by Bybee et al. (2015) and stated that the attempts to unite the use of terms naiad, nymph and larva should be "*rooted in the real origin of the terminological problem*", otherwise they create only more confusion. This root is not seen in the dissimilarity of the lifestyles and morphologies between immatures and adults, but in the presence of two different types of wing development in winged insects, external in exopterygote insects and internal in Endopterygota, and theories proposed to explain this.

Rédei & Štys (2016) recognized two main groups of these theories. First is the heterochrony theory, where the immature stages of exopterygote and endopterygote insects are seen as homologous, with hemimetaboly being the more plesiomorphic type of metamorphosis, while in holometaboly the development of adult features is delayed to later instars resulting in the presence of pupa. Hence the pupa of Endopterygota and the last immature instar of exopterygote insects can be seen as homologous, and therefore the terms larva and nymph are seen as equal (e.g. Hinton 1963; Kukalová-Peck 1983).

The second one is the pronymphal theory, where the immatures of Endopterygota represent the pronymph of exopterygote insects (intermediate nonfeeding stage between embryonal and postembryonal developmental stages that moults into the first instar shortly after hatching from

the egg). Therefore, the juveniles of hemimetabolous and holometabolous insects are not deemed homologous and special terms may be used to distinguish them (e.g. Berlese 1913; Comstock 1918; Heslop-Harrison 1958; Truman & Riddiford 1999; Erezylmaz 2006).

However, this question of homology of exopterygote and endopterygote immature stages is deemed rather philosophical by Rédei & Štys (2016), because it is very often impossible to homologize individual developmental instars of different insect groups, since their growth trajectories can differ considerably owning to heterochronic changes in the development of specific organs. Rejecting the pronymphal theory for a lack of evidence, the authors support the heterochrony theory, recently supported also by Jindra (2019) in the study of the timing of juvenile hormone signalling. In conclusion, reached also by other authors (e.g. Tillyard 1917; Sehnal 1996; Sahlen et al. 2016), they suggest the usage of the general zoological term larva, used for immatures of many other metazoan groups with indirect development, for all immature stages of insects (except pupa) as one possible solution. On the other hand, Rédei and Štys (2016) point out, that the terms nymph and naiad don't have a precise scientific meaning in the sense of homology and can be used to designate the morphological and ecological differences between the larval types.

For simplicity, in the present text we will use the term larva, to describe the immatures of all insects, but in the line of the thinking, the use of the terms nymph and naiad is also acceptable for the distinction of the immatures of hemimetabolan insects and aquatic immatures of mayflies, dragonflies and stoneflies respectively.

1.3. The wings of insects

The origin of insect wings represents one of the most significant events in the insect evolution. Insects were the first nature's active flyers and took off to the sky 400 million years ago (Engel et al. 2013), but the wings do not serve only the purpose of flight. The wings allowed for its carriers to discover and colonize new niches, to better escape or hide form its predators, to more easily find food or a reproductive partner, and maybe also to regulate their body temperature by changing the wing position and orientation (Grimaldi & Engel 2005; Engel 2015; Gullan & Cranston 2014).

Even though occasional voices claim, that the wings of insects must have evolved more times independently (La Greca 1980), in the present time it is widely accepted, that they emerged only once in the history of insects, based on the presumed groundplan of insect wing venation observable in all orders and shared features of the thoracic musculature (Klowden 2013; Belles 2020). Thus, the recent winged insects form a monophyletic group of Pterygota, which is considered as a sister group of Zygentoma and diverged in the Early Carboniferous or even before in the Early-Middle Devonian according to the calibrated phylogeny trees (Rehm et al. 2011; Misof et al. 2014; Wang 2016).

The oldest known direct evidence of winged insect bearing the fully developed meso- and metathoracic wings is *Delitzschala bitterfeldensis* Brauckmann and Schneider, 1996 (Palaeodictyoptera: Spilapteridae) (Brauckmann & Schneider 1996) from the lowermost Late Carboniferous. This species shows that the wings were already used for active flight. Therefore, it can certainly be assumed, that the origin of wings took place many millions of years before the dating of this fossil.

Older fossils of supposedly winged insects were discovered and described, for example *Strudiella devonica* Garrouste, et al. 2012 from the Late Devonian period, or *Rhyniognatha hirsti* Tillyard, 1928 from the Early Devonian (Garrouste et al. 2012; Engel & Grimaldi 2004), but these are fragmentary or poorly preserved and hard to interpret (e.g. Hörnschemeyer et al. 2012; Haug & Haug 2017). Thus, the fossil record that would clarify the origin of wings in insects stays rather incomplete. To answer the questions about their first appearance one must look for the evidence elsewhere at this moment. One of the possible directions is the study of developing wings and comparison of these characters between recent and fossil representatives of winged insects.

1.3.1. The origin of insect wings

Two main approaches emerged to explain the origin of insect wings in the past. The first one assumed, that the wings appeared as new structures without relation to any pre-existing organs. This view was strongly influenced by the creationism and the idea of special creation and was soon rejected. The second approach, that the wings developed from a pre-existing structure, became fully accepted. From the numerous hypotheses formulated within this concept, two major groups prevailed (Crampton 1916).

1.3.1.1. Tracheal gills hypothesis

The first of the two main hypotheses about the wing origin from pre-existing structure is that of development of insect wings from tracheal gills. The roots of this concept date back to 1809, when Lorenz Oken proposed the emergence of flying insects from aquatic environment and later in 1831, when he suggested the origin of wings from modified tracheal gills (Oken 1809, 1831). Carl Gegenbaur (1878) proposed that the wings could be derived from dorsal tracheal gills homologous with the tracheal gills on the body of modern aquatic insect larvae. In this view, the gills would enlarge for better gas exchange. Later in a semi-terrestrial insect, the enlarged gills could have been used for gliding or steering and finally with the simultaneous advancement of necessary articulation and musculature, they evolved into wings capable of active flight. Woodworth (1906) suggested, that the tracheal gills themselves were too delicate to serve as a precursor of the wings in such a way, and that the wings could have developed from their subunit, like the rigid planes covering the gills in some mayfly larvae.

Wigglesworth (1973) proposed the homology of insect wings with abdominal tracheal gills in larvae of recent mayflies, which are in some species used for cover, ventilation or even as paddles. Since the abdominal tracheal gills of mayflies are regarded as homologous to abdominal styli of Archaeognatha, which are supposedly remnants of the abdominal appendages, the insect wings should be derived from pleural thoracic appendages or their subunit. The ancestral tracheal gill covers could have evolved into paddles in the aquatic insects. Once they became semi-aquatic, the enlarged plates could help the insect to be carried by wind, and if the insect achieved the ability to control the flight, these plates could then develop into flapping wings.

This thought of wings origin from pleural thoracic appendage was further elaborated by Kukalová-Peck (1978, 1983), who proposed the well-known exite origin hypothesis, where the wing should be homologous with the exite of epicoxa of crustacean leg. The author supports

this claim by the position of the wing on the thorax between the two basal leg articles integrated into the pleura, epicoxa and subcoxa, and hypothesized, that the wing and axilla with articulation of sclerites could have developed from the epicoxa and exite of the subcoxa of the thoracic appendage. This hypothesis was also supported by Averof & Cohen (1997), who provided evidence for the wing origin from epipodites, dorsal gill-like branches of multibranched appendages of aquatic ancestors of crustaceans (and insects). In their developmental genetic study, the authors showed, that the gene expression patterns in crustacean appendages and insect wings are identical.

Another relevant study is that of Marden & Kramer (1994), where the authors based their reasoning on the observations of water-skimming behaviour of some extant stoneflies. The authors suggest that the surface-skimming could have been a step in the evolution of wings, when from aquatic insect with mobile gill covers arose a semi-terrestrial or terrestrial imago with small winglets, that allowed to skim across the water surface. Since the fastest skimmers would be the most successful, the selection would favour longer wings and stronger wing muscles, which could eventually lead to active flight.

1.3.1.2. Paranotal lobes hypothesis

The second of the hypotheses of the wing origin from pre-existing structure is the hypothesis of development from outgrowths of the tergum, the thoracic winglets or paranotal lobes. This thought was firstly introduced by Friedrich Müller (1873) based on his study of termites and was further elaborated by numerous authors (e.g. Comstock & Needham 1899; Comstock 1918; Snodgrass 1935; Hamilton 1971; Kristensen 1975). Müller (1873) stated, that the paranotal lobes on the thorax of termite larvae are incipient wings, and since the trachea did not penetrate them, they could not be evolved from tracheal gills.

The work of Crampton (1916) became the critical point in the discussion of the insect wing origin, where all evidence in support of both major hypotheses was evaluated and the paranotal theory gained the support, which was kept for a long time. The arguments against the tracheal gill hypothesis included the aerial respiration being probably the primary type in insects, meaning that the recent insect forms are all descendants of terrestrial ancestors and, therefore, the wings could not be of the tracheal gill origin. However, the author himself pointed out the possibility that the ancestors of all winged insects could became aquatic for a while and acquired tracheal gills, which then developed into wings when the insects became terrestrial again.

Some of the evidence proposed for the paranotal hypothesis in Crampton's work include (1) the widespread presence of leaping behaviour and paranotal lobes on the prothorax of many extant insects including numerous immature forms of some insects, and (2) fusion of the upper surface of larval wing pads with the thoracic terga. Hamilton (1971) suggested, that the lateral tergal lobes in Archaeognatha and Zygentoma closely resemble the proposed paranotal lobes of pterygote ancestor. He also developed a model of hypothetical pattern of tracheation and vein precursors in the paranotal lobes of the pterygote ancestor, which were supposedly matching the pattern found in the prothoracic lobes of some Palaeozoic representatives as Palaeodictyoptera.

In the recent insects the wings are present only in two pairs on the meso- and metathorax of adults, but in Palaeodictyoptera and some other Palaeozoic insects (as Geroptera) there was present another pair of wing-like appendages on the prothorax. Although, unlike the meso- and metathoracic wings, these winglets never fully developed into organs for active flight, some authors (Snodgrass 1935; Hamilton 1971) assumed, that the wings could developed from similar lobes of the meso- and metathoracic notum.

One of the presumed original functions of the paranotal lobes could have been coverage of the basal portions of legs (Crampton 1916) or thermoregulation (Whalley 1979; Douglas 1980; Kingsolver & Koehl 1985; Kukalová-Peck 1987), so that the bigger the lobes, the quicker the sun basking insect warmed up. Another suggested option is the use of winglets in mating behaviour (Alexander & Brown 1963), where they could serve as organs of enigmatic display. Nevertheless, these enlarged outgrowths could then become useful for gliding from elevated places or in leaping insects and then steering (Crampton 1916; Flower 1964; Staniczek et al. 2014), and eventually become the flapping wings capable of active flight with simultaneously evolved articulation with the body wall and the musculature necessary for the wing movements (Pringle 1957; Forbes 1943; Hamilton 1971).

Kukalová-Peck (1978), however, stated, that the energy-efficient act of gliding in recent insects is not that trivial, as it is achieved by combination of complex wing articulation with the body wall and mechanism for keeping the wings extended, and has, thus, evolved through many adaptations. Kukalová-Peck (1983) further claimed, on the basis of the previous studies (Sharov 1957, 1966, 1973; Carpenter & Richardson 1969; Kukalová 1968; Wotton 1972), that the larval wing pads of Palaeozoic pterygote insects were primarily fully articulated and movable and that the fusion of wing pads with notum in todays' larvae is secondary and is the result of adaptation for life in water streams.

1.3.1.3. Contemporary view of the wing origin

More recently, Niwa et al. (2010) introduced dual model of insect wing origin linking both above mentioned hypotheses together. In their study of gene expression in mayflies and bristletails they discovered that the wings emerge as composite structures of the tergum and leg-derived elements of pleura. This model is also supported by numerous evo-devo and genetic or genomic studies on other taxa (Clark-Hachtel et al. 2013; Medved et al. 2015; Linz & Tomoyasu 2018; Almudi et al. 2020) (Fig. 2). Elias-Neto & Belles (2016) found out, that the winglessness of the prothorax is determined by the gene *Sex combs reduced* (*Scr*) in *Blattella germanica* (Linnaeus, 1767). Also, by interference of *Scr*, ectopic wing structures form on the prothorax of the cockroach from the expansion of the latero-posterior region of the pronotum with contribution of the proepimeron. Prokop et al. (2017a) supported this hypothesis with paleontological data, when they showed, that the palaeodictyopteran wings were articulated only in the medial area (contrary to previous claims of Kukalová-Peck, 1983), but they were anteriorly and especially posteriorly broadly fused with notum.
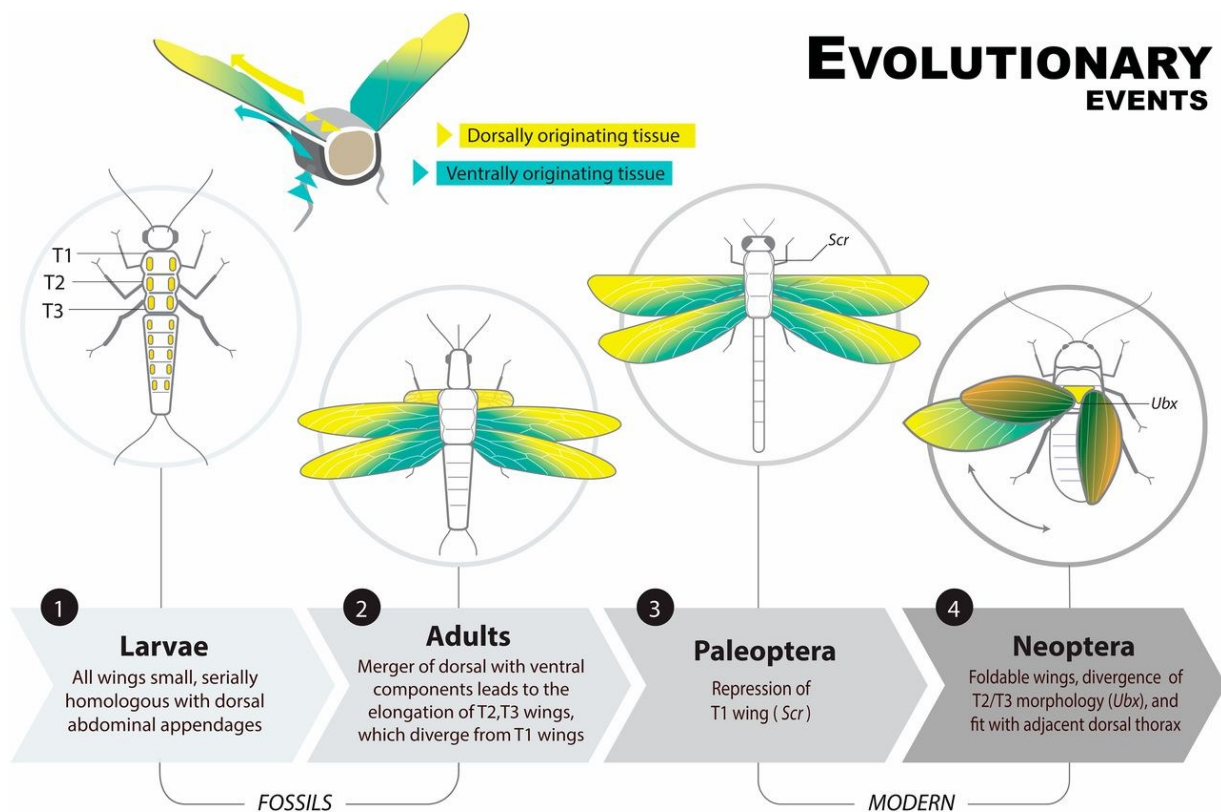

Figure 2: Major events in the divergence and segmental diversification of insect wings from the genetic point of view: expression of the genes *Scr* (*Sex combs reduced*) and *Ubx* (*Ultrabithorax*), which are responsible for the winglessness of T1 (prothorax) and differentiation of T2 (mesothorax)/T3 (metathorax), respectively (Medved et al. 2015).

1.3.2. The structure of insect wings

The fore and hind wings of adult modern Pterygote insects are formed from two closely placed cuticular membranes and supported by a framework of longitudinal veins and transverse cross-veins, cuticular thickenings of each membrane. The epidermal layers of the two membranes of the adult wings are usually degraded, so the membranous parts of wings appear entirely cuticular. The tracheae, nerves and active haemolymph flow run mainly in the wing veins, however, in some cases, the nerves and tracheae pass between the two not completely united wing walls (Arnold 1964).

The wing can be divided into three main areas. The first one, remigium, is the most anterior part of the wing that has the main role in flight. In this region we can find a majority of the longitudinal veins (from costa to cubitus). The second one is clavus, vannus, or anal area, when expanded in the hind wing, which is often separated from remigium by vannal or claval or furrow and is divided from the last area, the jugum, by a jugal fold (Wootton 1992; Chapman et al. 2013; Beutel et al. 2014) (Fig. 3).
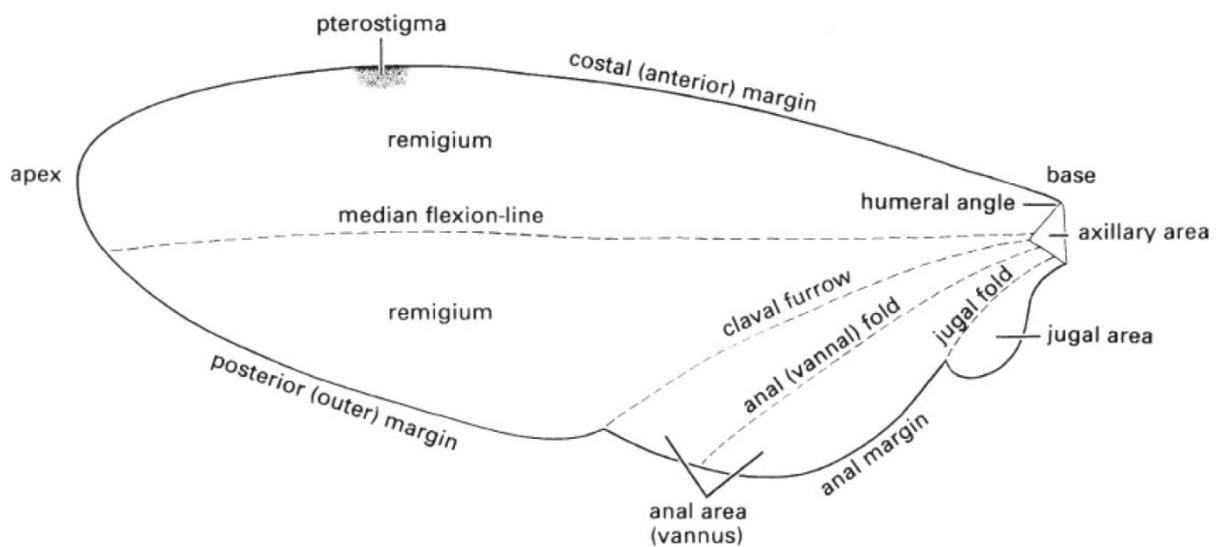
Figure 3: The main areas, folds and margins of a generalized insect wing (Gullan & Cranston 2014).

1.3.2.1. Articulation of the wings with body

At their base the wings articulate with the scutum and pleura of adult insect's body via the complex of movable articular sclerites in the so-called axillary area. The main types of insect wing articulation are those of the Ephemeroptera, the Odonata and the entire group Neoptera. Homologies of these varied sclerite schemes among the distinct basal lineages of the Pterygota

still remain ununified and many authors have addressed the questions of the wing base types origin and derivation (e.g. Hamilton 1972; Kukalová-Peck 1978, 1983; Yoshizawa & Nynomyia 2007; Willkommen & Hornschemeyer 2007a, b; Hassenfuss 2008; Willkommen 2008, 2009; Ninomiya & Yoshizawa 2009).

Kukalová-Peck et al. (2009) interpreted the wing articulation of the Diaphanopterodea (Palaeodictyopterida) as the most primitive one. The hypothetical groundplan of the wing articulation is associated with the origin of wing from leg-like appendage, supported by the author, and the sclerites are derived from fragments of the limb. The wing sclerites were presumably arranged in eight rows and three columns, proxalaria, axalaria and fulcalaria, and each row was articulated to the fourth column made up of sclerotized blood sinuses, the basivenalia. Each row of sclerites was associated with one main longitudinal vein, precostal, costal, subcostal, radial, medial, cubital, anal and jugal. According to this hypothesis, the articulation patterns of all known insects arose by fusion, reduction and other modifications of this fundamental plan (Fig. 4).
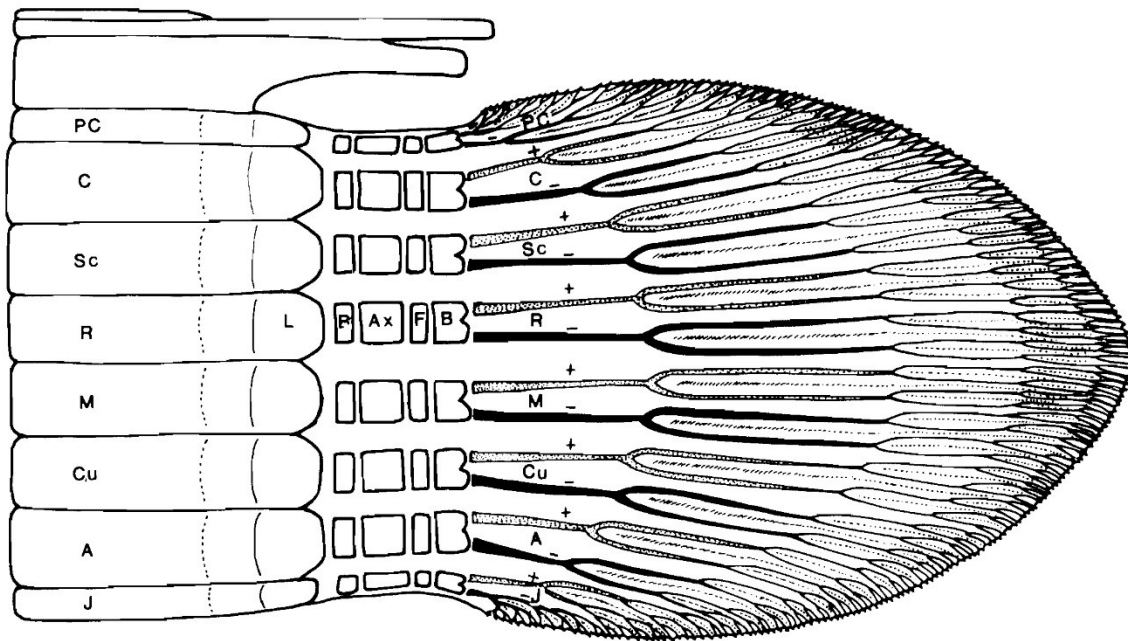
Figure 4: Hypothetical model of articulation and wing venation groundplan of Pterygota according to Kukalová-Peck (1983). +, convex anterior; -, concave posterior; A, anal; Ax, axalaria; B, basivenalia; C, costa; Cu, cubitus; F, fulcalaria; J, jugal; L, tergal lobes; M, media; P, proxalaria; PC, precosta; R, radius; Sc, subcostal.

In Paleoptera, which representatives cannot fold their wings over the abdomen, the articulation is formed by only two sclerites, the humeral and the axillary plate, in Odonata and by three

axillary sclerites in Ephemeroptera. Also, Neoptera have wings articulated by three sclerites, with the exception of Orthoptera, Hymenoptera and Diptera with four axillary sclerites (Fig. 5).
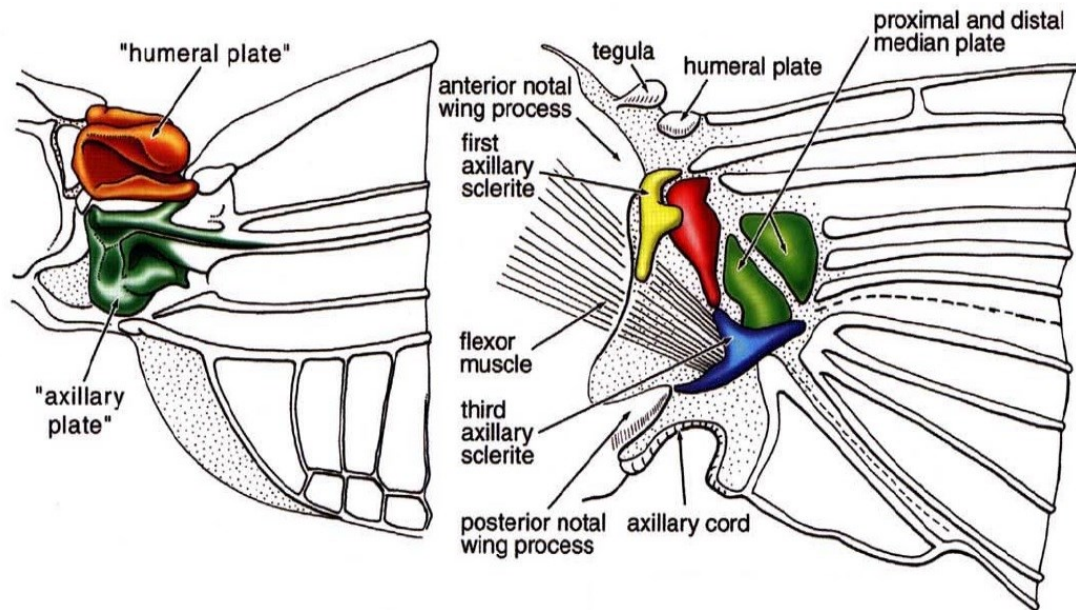
Figure 5: Wing articulation in Palaeoptera (Odonata) (left) and generalized Neoptera (right) (Grimaldi & Engel 2005).

1.3.2.2. General characters of insect wing venation

The adult insect wings bear prominent longitudinal veins formed by the separated membranous layers of the wing and thickened cuticle. These hollow tubes run from the wing base to the wing tip and contain haemolymph, tracheae and nerves (Comstock 1918) (Fig. 6).

Figure 6: Diagrammatic section through part of a wing including a transverse section of a vein (Chapman et al. 2013).

The longitudinal veins can be corrugated, as is found in some Palaeozoic and some Mesozoic insects and in recent Ephemeroptera and Odonata (Spieth 1932; Holdsworth 1942; Kukalová-Peck 1978). This means, that the veins are formed either in the dorsal or ventral membrane,

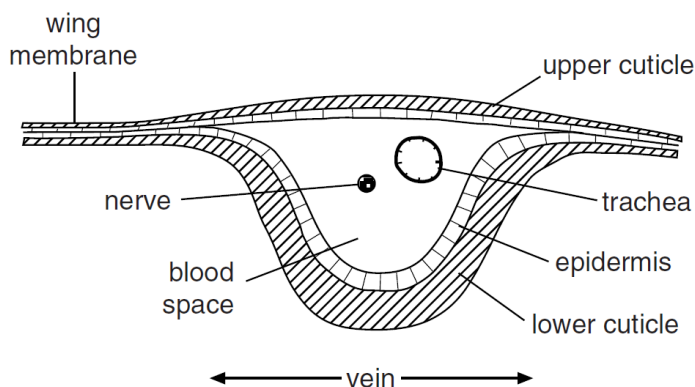leaving them alternatively raised and sunken with respect to the wing membrane (anterior branches usually convex and posterior branches concave) (Kukalová-Peck 1991), so that the wing resembles a fan and has increased mechanical stability (Spieth 1932; Beutel et al. 2014). In the neopterous insects wings, however, the veins are imprinted on both wing membranes and the corrugation is lost. In the ontogenetic development of *Pteronarcys proteus* Newman, 1838 (Plecoptera) the lacunae (vein-preceding cavities) of the future main longitudinal veins are more associated with the dorsal epithelium of the growing wing pad (Holdsworth 1942). Even the subcosta, being the only vein that is concave in adult *Pteronarcys* wing, is impressed on both membranes. Thus, in the neopterous insect orders the corrugation became lost, with the wings occurring as a flat plane (Fig. 7).
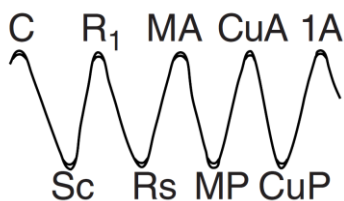
Figure 7: Scheme of a section through a wing showing the convex and concave veins. 1A, anal; C, costa; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; R_1, radius anterior; Rs, radius posterior; Sc, subcosta (Chapman et al. 2013).

The thickness of vein walls and shape of vein cross-sections varies between different insects, which results in different degrees of bending deformation resistance (Combes et al. 2010). Also, cross-veins play a significant role in supporting the wing plane. They improve the wing toughness by blocking the cracks and fractures of the membrane to propagate (Dirks & Taylor 2012) and they can also serve to strengthen some areas of the wing as support brackets. The cross-veins can also be annulated in some areas, which facilitates the wing's bending ability (Combes et al. 2010).

The cross-veins and longitudinal veins together form a system of cells, areas of wing membrane surrounded by veins. These cells are usually named after the anterior bordering vein and they can be fully closed from all sides or they may be open to the wing margin (Chapman et al. 2013).

1.3.2.3. Tracheation of the insect wings

The tracheal system of the insect's body serves for the transfer of oxygen to organs and muscles. The openings of the system, called spiracles, are located on the pleural areas of the body segments. The system is composed of tracheae, the invagination of the body wall lined with thin cuticular layer called intima, which bears the taenidia, spiral thickenings of the cuticle (Gullan & Cranston 2014). Before each moult the intima is laid anew and the old one is shed in the ecdysis. The tracheae branch and the ending most tiny branches are called tracheoles. They are blind, lack taenidia and end in target tissues (Beutel et al. 2014) (Fig. 8).
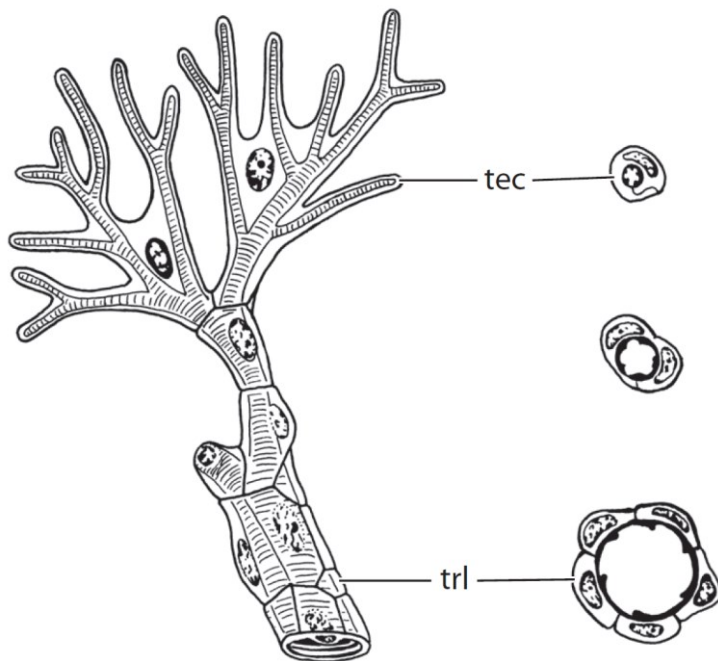
Figure 8: Distal part of trachea. tec, terminal cell; trl, tracheole (Beutel et al. 2014).

In the most general condition, the tracheal system of the wings is connected with three spiracles, first on the mesothorax, second on the metathorax and third on the first abdominal segment. The first and third spiracle provides one common tracheal branch to the leg and wing of meso- and metathorax respectively. The middle spiracle contributes two branches, one to the mesothoracic appendages and second to the metathoracic ones. The metathoracic spiracle is degraded in many insects, still its tracheal trunk is preserved and connected with branches to the thoracic appendages. Each wing is supplied by two tracheal trunks arising from the tracheal branch shared with the leg of its anterior and posterior spiracle. The anterior tracheal branch of the wing divides into tracheae supplying the costo-radial area of the wing (including the medial trachea), and the posterior tracheal branch divides into tracheae supplying the cubito-anal area. The anterior and posterior tracheal groups can be separated, as in *Pteronarcys* (Plecoptera) or, as in most insects, connected by the basal transversal trachea. In Ephemeroptera, the posterior

tracheal trunk is much reduced, with the cubito-anal tracheal group connected with the costo-radial group, and the connection of the posterior tracheal trunk to the tracheal branch of the posterior spiracle lost (Comstock 1918) (Fig. 9).
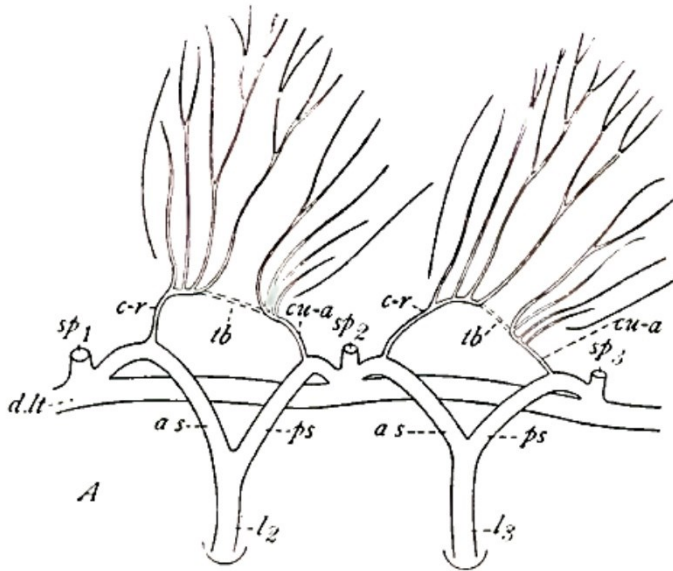

Figure 9: Basal connections of tracheae (Comstock 1918). as, anterior stem of the leg trachea; c-r, costo-radial trachea; cu-a, cubito-anal trachea; d.lt, dorsal longitudinal trachea l2, mesothoracic leg trachea; l3, metathoracic leg trachea; ps, posterior stem of the leg trachea; sp1, mesothoracic spiracle; sp2, metathoracic spiracle; sp3, first abdominal spiracle; tb, basal transversal trachea.

1.3.2.4. The relationship between wing venation and tracheation

The endeavour for a united terminology of insect wing venation in all insect orders has been going on for over a century (e. g. Redtenbacher 1886; Comstock & Comstock 1895; Comstock 1918; Lameere 1923; Kukalová-Peck 1978) and yet still the homologization of wing venational systems together with previously mentioned articulation types in different insect orders remains understudied and blurred. The description and attribution of the veins varies with different authors and the historically established diverse naming systems for different insect orders are hard to update simultaneously (Breitkreuz et al. 2017).

The vein naming systems used today are mainly based on the work of Redtenbacher (1886), where he distinguished five convex veins named costa, radius, media, cubitus and anal (raised above surface); and up to seven concave veins (sunken below surface), with only one vein named, the subcosta. The convex and concave veins were placed alternately on the wing. Together with later authors (e.g. Spuler 1892), Redtenbacher did not consider the costa as vein, but as a mere thickened edge of the wing.

Redtenbacher's system was later revised by Comstock (1918) in his monography "The Wings of Insects", where the author refused the theory of alternating convex and concave wing veins and the assumption that the first insect wings had bigger number of longitudinal veins, which

decreased with the reduction of abundant veins. Since Comstock believed, that the tracheae determine the position of the main longitudinal veins, all known insect wing venation patterns should have arisen by the modification of his primitive pattern of tracheation (Fig. 10) and thus the wing veins of all insect orders should be homologizable. This hypothetical model relies on the assumption, that the ancestral wing had as many tracheae as veins and that they were concurrent. In this model the wing was supplied by two tracheal branches, the anterior with costal, subcostal, radial (divided into radial and sector, today's radial anterior and radial posterior) and medial tracheae, and the posterior branch with one cubital and three anal branches.
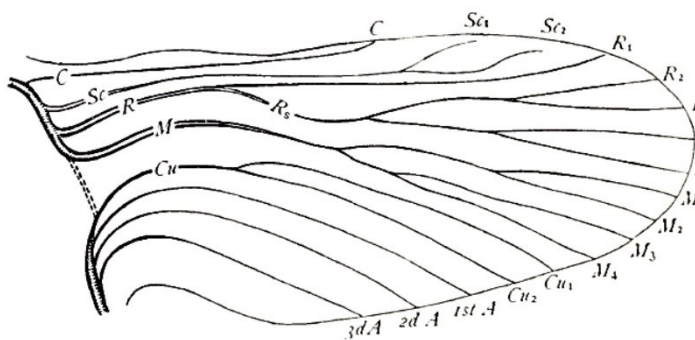

Figure 10: Hypothetical venation of a wing of the primitive nymph (Comstock 1918). A, anal; C, costa; Cu, cubitus; M, media; R, radius; Sc, subcosta.

Comstock (1918) attached a taxonomic significance to some features of the wing pad tracheation, especially the presence or absence of the transverse basal trachea. He divided insects based on this character. The first group contained Plecoptera and Blattodea, where the wing pad was supplied by two distinct groups of tracheae, the anterior costo-radial (including medial trachea) and posterior cubito-anal group. The second group was formed by all other insects, where the two tracheal groups were connected by the basal transverse trachea [in fact, the wing pad is tracheated by the branches of a longitudinal tracheal trunk, formed by the fusion of the anterior and posterior tracheal trunk (Whitten 1962)], and the medial (and sometimes even radial) trachea tend to migrate [according to Whitten (1962), migration or fusion of this tracheal branches is not possible and this fact can be explained as reduction of old trachea and arising of new trachea in next instar] towards the cubito-anal tracheal group. An exception is represented by Ephemeroptera wing pad, where only the anterior tracheal trunk is present [opposite to Comstock's view, Whitten (1962) argued, that the cubital and anal tracheae are of anterior tracheal origin]. Also, in some Trichoptera (Comstock 1918) and Diptera (Whitten 1962), the posterior trachea is secondarily reduced.

Beck (1920) attempted to verify some conclusions made by Comstock (1918) regarding the taxonomic significance of the wing pad and adult wing tracheation. He found, that in numerous specimens within one species, here *Blattella germanica*, the wing pattern of tracheation can be greatly instable. The media was either part of the anterior costal-radial stem, or, in another specimen, the media, and radius as well, migrated towards the cubito-anal stem, and in one case the transverse basal trachea was even completely lost.

Since the wing venation of the recent insect forms underwent many modification in the evolution, like fusion, loss or secondary gain of veins, it was not always possible to homologize the veins using the presumed archetypal venational pattern of Comstock, since it has been proposed on the basis of tracheation in recent insect larvae (Tillyard 1915, 1922; Lameere 1923; Snodgrass 1935). Moreover, the tracheation is not uniform even within single insect species, as showed by Beck (1920), and in some representatives of Holometabola, the irregularity of wing tracheation is even more pronounced (Ross 1936). Further, for example in Heteroptera, many of the longitudinal veins of adults do not have a corresponding trachea in the larval wing pad (Hoke 1926). It was necessary to find another way to clarify the wing vein homologies.

When trying to solve the problem of existence of the posterior branch of radius in the suborder Zygoptera (Odonata), Tillyard (1915, 1922) found, that based on the pattern of tracheation present in larvae, the particular vein in question would appear to be of complex origin, evolved through a complicated process of vein fusions. But when he compared the present state with the fossil Protodonata and Odonata, it was apparent, that the supposed posterior radial vein is a branch of media. Lameere 1923 also stressed the importance of the study of the Palaeozoic insects, since here the venational pattern should be preserved in the most complete form we can find. He noticed the alternation of raised and sunken veins in the Palaeozoic palaeopterous insects and Palaeodictyopterida and brought back the idea of vein corrugation. He suggested modifications of the Comstock's scheme in this way: each main vein has high (convex) and low (concave) branch (first proposed by Adolph 1879); subcosta is the concave posterior branch of convex costa; there are two main anal veins, first anal vein with posterior branch, and second anal vein with numerous posterior branches.

Another alternative to avoid the above-mentioned problems with wing vein homology based upon the larval wing pad tracheation, was proposed by Snodgrass (1935). He designed venation taxonomy based on the basal connections of the wing veins to the articulation sclerites of the wing, since the sclerites should have constant relations with each other and with the bases of the wing veins. The author noticed, that in some insects the presumed first anal vein is associated with cubitus at the wing base and is represented by a distinct trachea in the larval

wings of many hemimetabolous insects. Therefore, he distinguished it from other anal veins and called it the postcubitus (PCu). He also added the jugal lobe with one or two jugal veins to the general pattern of wing venation (Fig. 11).
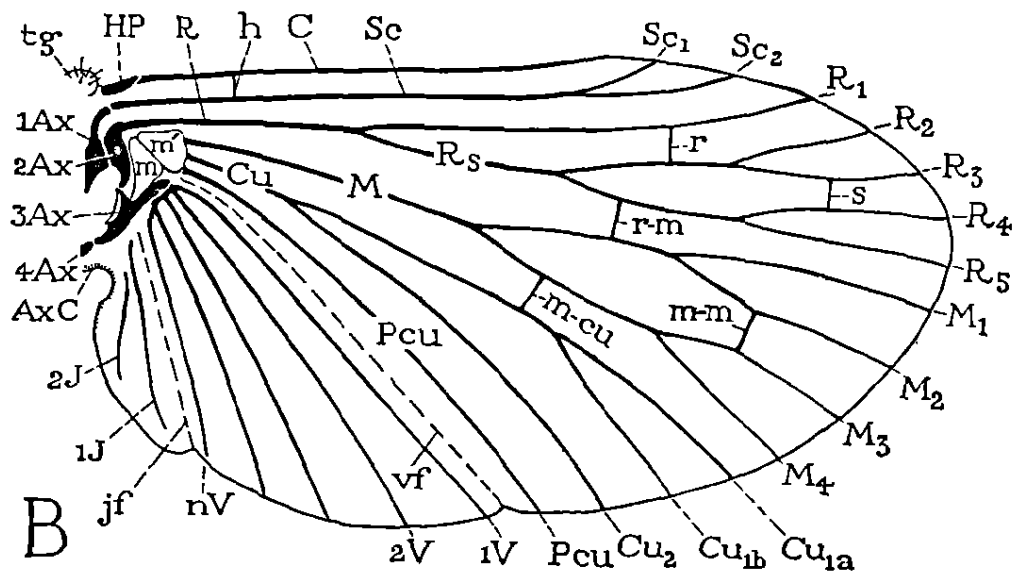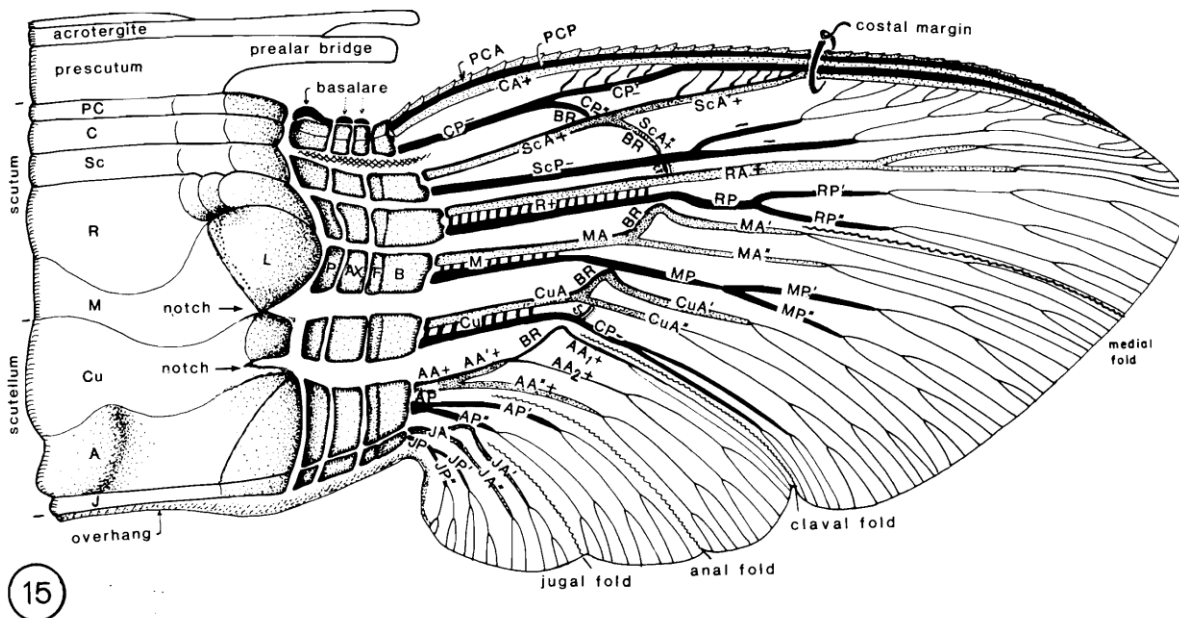

Figure 11: Wing venation and axillaries after Sondgrass (1935). A, anal; Ax, axillary sclerites; AxC, axillary cord; C, costa; Cu, cubitus; h, humeral cross-vein; HP, humeral plate; J, jugal; jf, jugal fold; M, media; m, m', median plates; MA, media anterior; m-m, median cross-vein; m-cu, mediocubital cross-vein; MP, media posterior; Pc, precosta; PCu, postcubitus (first anal); R, radius; r, radial cross-vein; Rs, radial sector; s, sectorial cross-vein; tg, rudiment of tegula; V, vannal veins (anal veins except the first; vf, vannal fold.)

The pretracheation theory was rejected, based on the observation of the development of wing pads in Plecoptera, where it was evident, that the vein lacunae (precursors of adult wing veins) appear earlier in development than the tracheae, which invade them (Holdsworth 1942). Another evidence for the inconsistency of the pattern of tracheation in wing pads and adult wings was provided in Heteroptera (Wigglesworth 1954; Leston 1963) and Blattodea (Smart 1956). In the lack of oxygen, the tracheation of the wing pad became less regular with increased number of bigger longitudinal tracheae, but the number of vein lacunae remained the same. In adult wings of insects reared in atmosphere with lower oxygen, some tracheae can be reduced, while the associated vein is only partially developed in places, where the surrounding tracheae managed to provide oxygen, by sending transverse tracheae to the lacuna. After an injury on the larval wing pad, the detracheated area is retracheated by additional growth of the preserved tracheae in the path of the vein lacunae, with the pattern of tracheation deviating from the general from. These results suggest that the predetermining factor for the venational pattern is

the system of lacunae and that adequate oxygen supply provided by tracheae is necessary for the development of adult veins.

More recently, Kukalová-Peck (1983, 1991) suggested that the groundplan of wing venation comprised of these eight main longitudinal veins arising from basivenal articulation areas: precosta, costa, subcosta, radius, media, cubitus, anal and jugal vein, and each vein had two sectors- anterior and posterior, which could merge in the course of evolution into the stem veins radius, media and cubitus. In this view, the outer wing margin is composed of joined precostal, costal and anterior subcostal veins (Fig. 12). Only recently Schubnel et al. (2020) supported the long-doubted theory of presence of an independent postcubital vein first described by Snodgrass (1935).

Figure 12: Model of composite flying wing with early changes towards asymmetry that accompanied flapping flight, after Kukalová-Peck (1983). +, convex anterior; -, concave posterior; A, anal; AA, anal anterior; AP, anal posterior; Ax, axalaria; B, basivenalia; BR, brace; C, costa; CA, costa anterior; CP, costa posterior; Cu, cubitus; CuA, cubitus anterior; CuP, cubitus posterior; F, fulcalaria; J, jugal; JA, jugal anterior; JP, jugal posterior; L, tergal lobes; M, media; MA, media anterior; MP, media posterior; P, proxalaria; PC, precosta; PCA, precosta anterior; PCP, precosta posterior; R, radius; RA, radius anterior; RP, radius posterior; Sc, subcosta; ScA, subcosta anterior; ScP, subcosta posterior.

Even though the tracheae are not predetermining the pattern of wing venation, they still may be useful, especially when placed in the context of evidence obtained by other approaches, as systemic characters and for identification of veins in many taxa (families, genera), where under normal conditions, the basic tracheal pattern is stable and correlates with the pattern of venation (Whitten 1962). For example, in cases where the change of course of a vein appears as a

formation of a cross-vein, but when correlated with pattern of tracheation, the two tracheae adjoining in the course of single vein reveal the true nature of the supposed cross-vein (Breitkreuz et al. 2017).

1.4. The postembryonic development of wings in recent insects

It was already mentioned by Malpighi (1687), that the wings of adult insects could be observed in their larval forms. Swammerdam (1738) showed for the first time, that the foundations of imaginal thoracic appendages in Diptera, Hymenoptera and Lepidoptera appear already in larva, not in pupa. These rudiments are located underneath the thoracic integument. Weismann (1864) carried out the first ever study of the internal processes of insect wing development in the Diptera, to find out that the dipteran larva bears six pairs of disc-like bodies, which he called imaginal discs, in its thorax. These bodies increased in size during the larval growth and some developed into adult legs and wings during the metamorphosis. These discoveries were followed by number of investigations of imaginal discs mainly in Diptera and Lepidoptera (e.g. Gonin 1894).

The first comprehensive study of insect wings development through many insect orders came out in the series of successive papers on "The Wings of Insects" by Comstock & Needham (1898, 1899), and later in famous monography "The Wings of Insects" published by Comstock (1918). In these accounts the internal changes in the wing pad development of hemimetabolous insects were thoroughly studied for the first time.

1.4.1. General features of insect wings development

The general morphology of the insect larva emerges in the process of organogenesis when the germ layers differentiate into organs and epidermis. The wing primordia are, as well as the other external organs, derived from the ectodermal germ cells (Belles 2020). In the less derived insects, the appendage primordia acquire the form of evaginations or wing pads on the outside of the developing embryo or larva. Only in part of the more derived holometabolous insects, some of the appendage primordia develop concealed in epidermal invaginations, which are usually called the imaginal discs (Gibson & Schubiger 2001). Comstock & Needham (1899) concluded, that despite the superficial differences of the postembryonic wing development in hemimetabolous and holometabolous insects, the basic developmental principle is common in both cases. The wing foundations in the larva have the structure of epidermal fold, formed by lower and upper epidermal layer, and filled with haemolymph, nerves, and tracheae. In the transformation of the last larval stage to adult, the epidermal layers almost completely dissolve, and the adult wing is predominantly cuticular structure. The active haemolymph circulation through the wing veins is however preserved in the adults (Snodgrass 1935; Arnold 1964).

1.4.2. Hemimetabolous insects

After hatching of the larva, the wing primordia can be found in some insects, e.g. Plecoptera, Heteroptera, as a "flange" or lateral expansion of the tergum, where epidermal cells change from squamose to columnar laterally (Holdsworth 1942; Nivedita 1981). In most species the wing pads appear later in the ontogenetic development. In some insects, as *Locusta migratoria* (Linnaeus, 1758) (Orthoptera), wing pads can be already present externally from the first larval instar (Berlese 1913; Snodgrass 1935). In some hemimetabolous insects, as in cockroaches, the wing primordia are enclosed in a cuticle pocket in the laterobasal part of the meso- and metathorax called the pteroteca (Belles 2020).

The wing pads of hemimetabolous insects arise as sac-like folds of the body wall and are located on the dorsolateral margin of the meso- and metathorax, at the separation of tergum and pleura. In most hemimetabolous insects, the extension of the wing pads in the larval development is somewhat gradual with wings growing progressively at each moult, with the growth increased in instars closest to transformation into the imago. Before each moult, the wing rudiment within the wing sheath becomes wrinkled, when the epidermal layers elongate, and this happens right at the time of the beginning of cuticle production. The epidermis of the vein region wrinkles only transversally, the membranous areas wrinkle in transverse and also longitudinal direction. The wing pads are fully fused with the notum and they are formed by two layers of epidermal cells on basement membranes continuous with the epidermis of the body wall and covered by the wing pad cuticle, also called the wing pad sheath. The epidermal cells of the dorsal and ventral layer are elongated vertically and their basement membranes touch and form a middle membrane. Later, cavities start to form between these basement membranes, forming the channels preceding the wing veins, the lacunae (Fig. 13). Later in development, tracheae and sometimes nerves enter these lacunae, but apparently not all lacunae, e.g. in Plecoptera the circumambient costal lacunae do not contain any tracheae or nerves. The tracheas inside lacunae give rise to bundles of tracheoles. In the last larval instar, the epidermal cells along the vein lacunae condense and become cuboidal, producing a thick cuticular layer, which will become the final wall of the wing veins. In this stage the wing veins are formed by opposing half-tubes, each half-tube formed by the one epidermal layer. The articulation sclerites appear after the moult to imago (Woodworth 1906; Comstock 1918; Snodgrass 1935; Holdsworth 1940, 1942; Nivedita 1981; Chapman et al. 2013).
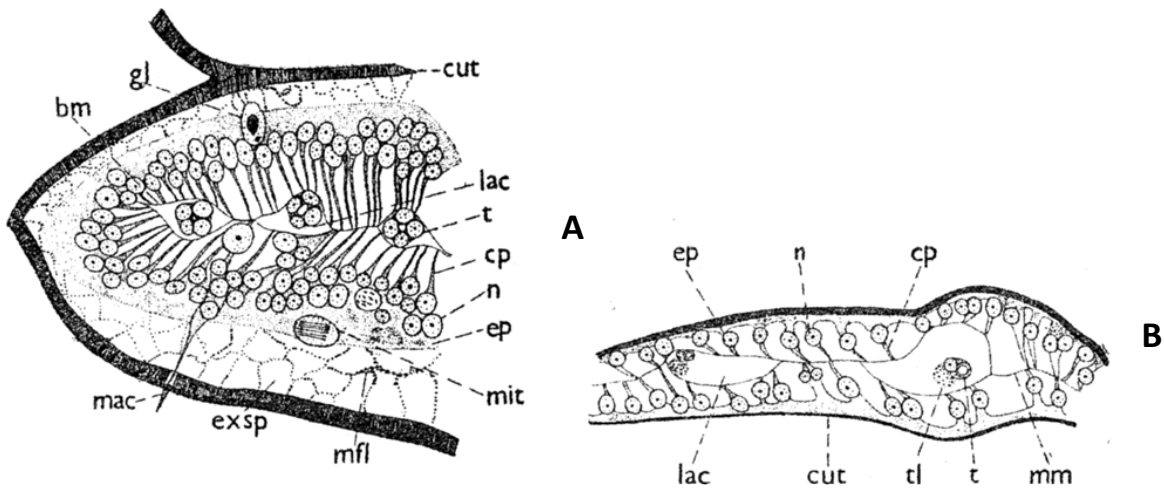
Figure 13: Section through the third instar (A) and fourth instar (B) larval wing pads of lygaeid *Oncopeltus fasciatus* showing lacunae and their respective tracheae (Nivedita 1981). bm, basement membrane; cp, cytoplasmic process; cut, cuticle; ep, epidermis; exsp, exuvial space; gl, gland cell; lac, lacuna; mac, macrotrichea; mfl, moulting fluid; mit, mitotic division; mm, middle membrane; n, nucleus; o, oenocyte; t, trachea; tl, tracheole; wr, wing pad.

The costal margin of the wing pads lies laterally and the posterior margin medially in relation to the thorax. But in Orthoptera and Odonata the wing pads lie in an reversed position, with the costal margin facing the thorax midline and the hind wing overlapping the fore wing, as the result of a rapid growth of the pleura, which then overlays the basal parts of the wing pads. The wing pads reverse to the usual position at the last moult and transformation into adult, where the costal margin of the folded wing lies ventro-laterally to the body (Comstock 1918, Chapman et al. 2013) (Fig. 14).
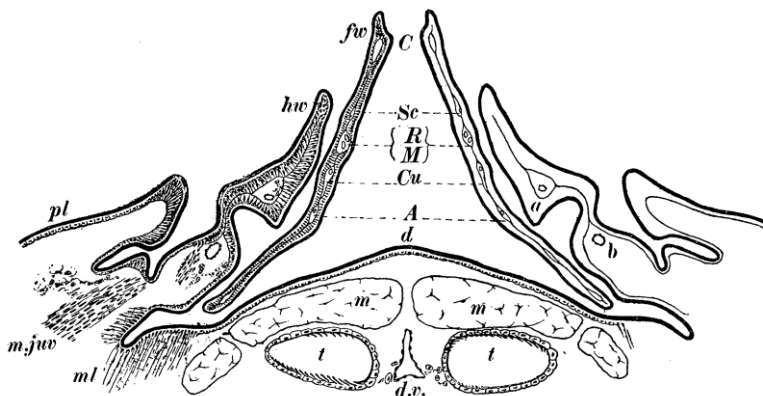
Figure 14: Dorsal part of a transverse section of a nymph of *Celithemis elisa*, one third grown (Comstock & Needham 1899). A, anal vein; a, b, the cut ends of the basal transverse trachea of the hind wing; C, costa; Cu, cubitus; d, dorsum; d.v., dorsal vessel; hw, hind wing; fw, fore wing; m, muscles in cross-section; m.juv, developing muscle of the wing; ml, muscles in longitudinal section; pl, pleurum; RM, the coalesced radius and media; Sc, subcosta; t, tracheae.

As stated before, only adult stages of the modern insects carry fully developed wings, with the only exception being the wings of subimago present in the order Ephemeroptera. The subimago represents developmental stage preceding the adult which poses functional wings and is capable of short flight. When moulting into the adult stage it sheds the cuticle of the wings (Sehnal et al. 1996; Gullan & Cranston 2014).

1.4.3. Holometabolous insects

Among more derived groups of holometabolous insects, as higher Diptera, Trichoptera and Lepidoptera, some Coleoptera, and some Hymenoptera, the external appearance of the wing rudiments is delayed to the pupal stage, and the larval wing rudiments, called imaginal wing discs, are concealed in epidermal pockets, called peripodial cavities, under the larval cuticle. Fine cuticular layer is lining the peripodial cavity and stalk, the epidermal layer connecting the wing disc with the epidermis of the body. This cuticular lining is dissolved before every ecdysis (Švácha 1992).

In the most studied insect in the regard of imaginal discs development as *Drosophila melanogaster* Meigen, 1839 (Diptera), the wing discs appear already in the early embryonal stage and are present at the time of hatching of the larva. Sometimes this condition is considered as common for all holometabolous insects, but *D. melanogaster* has very advanced and specialized larva, and the state of its wing development is probably very distant from the form of wing development in less derived holometabolans (Švácha 1992).

The imaginal wing discs are not present in the final larval (pre-pupal) instars of Megaloptera, Neuroptera, many Coleoptera, sawflies (Hymenoptera) and Mecoptera (Švácha 1992), and their absence in the final larval instar was previously described by Weismann (1866) in Culiciformia (Diptera). In these discs-lacking insects the wings develop externally in the last larval (pre-pupal) instar, for example in most Coleoptera (Comstock & Needham 1899, Tower 1903). Here the wing rudiment arises as evagination of the epidermis under the last larval cuticle.

Švácha (1992) proposed his hypothesis of the evolution of the holometabolous development from the hemimetabolous, where the wing development was delayed and shifted into the larval - pupal transformation. Thus, the ancestral condition is the one found in the less-derived Holometabola. The subsequent appearance of the imaginal wing discs in the more derived holometabolous insects allowed acceleration of the metamorphosis by restoring the possibility of the wing development to concur with the larval development. This condition evolved at least

four times independently, in some Coleoptera, higher Hymenoptera, some Diptera, and Trichoptera + Lepidoptera.

Švácha (1992) considered the wing development of *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionoidea) an intermediate stage between this ancestral external and derived internal development of wings in earlier ontogenetic stages. According to Quennedey & Quennedey (1990) the wing discs of *T. molitor* are developing during the last larval instar as epidermal thickenings located under the lateral margins of meso- and metathoracic tergites. After the thickened part of epidermis detaches from the cuticle, subsequently two grooves on the thickening begin to form. Between these two grooves an evagination occurs, forming the wing rudiment inside the peripodial cavity. The cells of the two layers of the wing rudiments proliferate rapidly. The wing rudiments grow ventrally while they move dorsally, the two epidermal layer cells elongated and densely packed forming lacunae containing tracheae and haemolymph. Close to the end of the last larval instar, the epidermal cells of the wing rudiments begin to flatten, resulting in highly crumpled appearance of the wing rudiments because of the limited space. After this the epidermis of the whole body deposits new cuticle, and after the pupal ecdysis, the wing rudiments unfold. Comstock & Needham (1899) studied the development of imaginal wing discs in coccinellid beetle *Hippodamia tredecimpunctata* (Linnaeus, 1758). These discs originate as a thickening of the epidermis in early larval stages, but the general processes of the later development seem identical to that of *T. molitor*.

In *Pieris rapae* (Linnaeus, 1758) (Lepidoptera: Pieridae) the wing imaginal discs appear already in the embryo as thickening of the epidermis on wing bearing segments, but invaginate in the second larval instar to form the peripodial cavity. In the third instar the evagination of the wing rudiment begins. During the fourth larval stage, the wing rudiments begin to grow in the peripodial cavity. Near the end of the fifth and last larval instar the wing rudiments are pushed out of the peripodial cavity, become external, lying under the last larval cuticle. After the pupal ecdysis, the wing rudiments are wholly external, fixed to the pupa. After the last moult, they expand into the adult wings (Comstock 1918).

In the *D. melanogaster* the wing development begins already in the early stages of embryogenesis, whit the invagination of imaginal discs. Each disc begins as a group of around 50 cells of epidermal monolayer, and grows rapidly during the larval development, so that in the last larval instar the folded epidermal layer contains thousands of cells (Fristrom & Rickoll 1982). Although the extension of the wing rudiment in the peripodial cavity is restricted by the lack of space, cell division takes place here (Comstock & Needham 1899; Snodgrass 1935). The wing discs growth of *Drosophila* has been found to be dependent on the moulting cycle of

the insect, and the cell division decreases at every larval moult (Garcia-Bellido & Merriam 1971).

When the larva of *Drosophila* hatches, the invagination of the peripodial cavity is already finished. The wing evagination itself begins to form in the second larval instar and grows more extensively in the third larval instar (Chapman et al. 2013). The wing discs are then formed by the columnar cells of the wing rudiment epidermis and squamose peripodial cells of the peripodial cavity. In the middle of the third instar, the hormone ecdysone induces the wing rudiment elongation and then eversion, when the peripodial epithelium contracts, which pushes the wing rudiment out of the peripodial sack and larval epidermis (Milner et al. 1983; Fristrom & Fristrom 1992; Gibson & Schubiger 2001) (Fig. 15). The peripodial epithelia contraction with subsequent wing rudiment eversion is probably general feature in other holometabolous groups with wing discs. When the wing rudiment of *Drosophila* emerges at pupation, the neighbouring everted wing discs spread and fuse along the dorsal midline, forming a continuous adult epidermis (Gibson & Schubiger 2001).
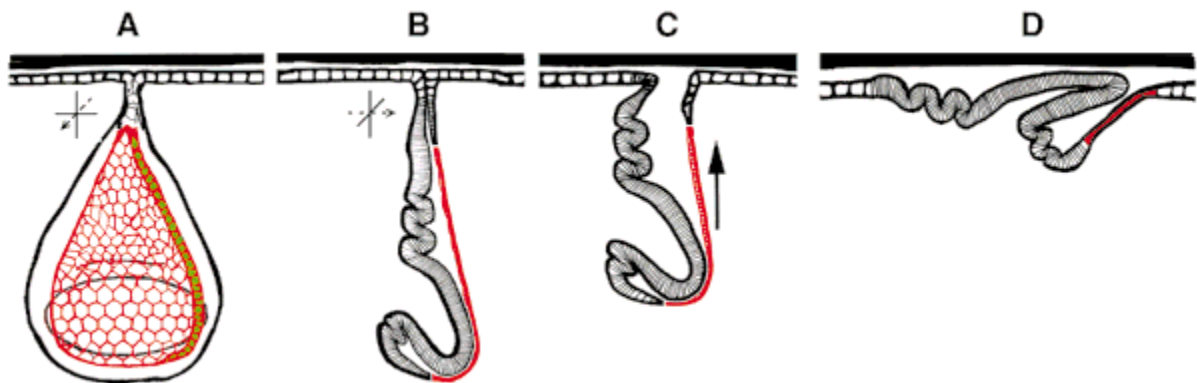
Figure 15: Wing disc eversion. A: The traditional two-dimensional view of a wing imaginal disc usually does not represent the squamous peripodial membrane cells, which are shown here in red. The green cells are peripodial "medial edge" cells. B: In cross section, the peripodial membrane is apparent (red), as well as the lumenal cavity between the two cell layers. C, D: During disc eversion, peripodial cells contract, forcing the columnar epithelium to the outside of the body (Gibson & Schubiger 2001).

Four veinal lacunae are already present in the wing rudiment, with the second one bifurcated distally. A nerve and trachea enter only the second lacuna. After the pupal cuticle is laid down, the upper and lower surface of the wing rudiment are forced apart by increased haemolymph pressure. The epidermal cells become stretched out into thin threads, with connections of the two surfaces broken everywhere, except the wing margins. After the inflation, the wing rudiment flattens again form the edges inward with the two epidermal layers pressing together.

The cell layers remain separated only in some lines, from which form the final wing veins. The vein lacunae become narrower with wing expansion and cell division. The cells above wing veins become crowded and columnar, while in other parts they are flattened. The development is complete when the adult cuticle is secreted by the epidermis (Chapman et al. 2013).

As for the tracheation of the evolving wing rudiments, in Holometabola these are located near thick tracheal trunk extending between the mesothorax and metathorax, and the metathoracic and first abdominal spiracle. The wing rudiments are first pervaded by few elongated cells of the tracheal epithelium, containing tracheoles, which are yet not connected to the main tracheal trunk. After a moult, the tracheoles connect with the trachea and at the same time extend in clumps into the vein lacunae of the wing rudiment. Some of the final wing tracheae are formed during the last larval instar and some during the pupal stage and enter the vein lacunae along the tracheoles. Since the opening of these new tracheae is closed by the intima of the main tracheal trunk, these become functional after the next moult. In the last larval stage, the wing rudiment is still supplied by air from the primary tracheoles. The newly formed tracheae become functional after the pupal or adult ecdysis, while the primary tracheae and tracheole deteriorate. The secondary tracheole bundles develop from the walls of the definitive tracheae, and these tracheoles become functional at the transformation to adult (Comstock 1918; Snodgrass 1935; Whitten 1962).

1.4.4. Historical account of the study of wing venation and tracheation

In the early stages of the research upon the development of wing pad tracheation and venation, the prevailing idea was, that the tracheae enter the wing pads before the formation of wing veins begins, known as the pretracheation theory, and that these tracheae then determine the later position of the main wing veins, which form around them later during development (Brauer & Redtenbacher 1888; Spuler 1892). This idea was widely accepted and supported by Comstock (1918) after observing the wing development and morphological transformations of insect wing tissues in numerous representatives of different orders of insects. Comstock (1918) assumed that the epidermal layers of each wing membrane, dorsal and ventral, are primarily separated in the earliest stages of wing development. Later, when the tracheae begin to protrude through the developing wing rudiment, the two epidermal layers begin to fuse in the areas between the protruding tracheae. After the vein lacunae delimitation by the merging epidermal layers is complete, the thickened lines of cuticle produced by the epidermal cells form the wing vein walls. In this sense, the tracheae would determine the position of the longitudinal veins in adult

wings, but Comstock admitted that there exist some differences between the larval wing pad tracheation and imaginal wing venation, the later adapted for the need of the adult's life. Comstock also saw a problem with application of this scheme in holometabolous insects, since there he observed that the tracheae enter the developing wing rudiments after the main vein lacunae are formed.

Woodworth (1906) in his influential paper entitled "The Wing Veins of Insects" described the arising wing veins from early stages of the wing pad development, as a series of separations between the wing pad's epidermal layers, visible as pale bands due to haemolymph transparency. In some insects tracheae enter these vein lacunae later in development, in others it seem that tracheae are present before the appearance of the vein cavities, but still the author assumed, that the vein lacunae have been already established before the trachea entered the cavity. Marshall (1913) came to the same conclusion after studying the development of wings in Trichoptera.

Many authors tried to understand the mechanisms underlying the developmental patterns of wing tracheation and venation (Beck 1920; Tillyard 1922; Lameere 1923; Snodgrass 1935; Wigglesworth 1954; Smart 1956; Whitten 1962; Leston 1963; see chapter 1.3.2.4.). The general conclusions were that the tracheation of the larval wing pads can be quite instable within single species, between consecutive larval instars or between larva, pupa, and adult, even between representatives of the same larval instar.

Finally, the pretracheation theory was rejected by Holdsworth (1940, 1942), who observed the whole development of the wing pads of *Pteronarcys proteus* Newman, 1838 (Plecoptera) from the first to the last larval instar. He concluded that the vein lacunae, which differentiate progressively from the second instar, are formed before the tracheae enter the wing pad and, therefore, the tracheae probably grow in "the paths of least resistance".

1.5. Evidence for postembryonic development in Palaeodictyopterida

The superorder Palaeodictyopterida makes up approximately half of the so far known Palaeozoic insect species (Grimaldi & Engel 2005). This extinct herbivorous group can be characterized by piercing-sucking mouthparts in the form of prolonged beak (rostrum), in number of species a pair of prothoracic lobes (mainly in Palaeodictyoptera), two pairs of permanently outstretched wings (except Diaphanopterodea) and long multisegmented cerci (Grimaldi & Engel 2005). The whole group is generally placed among the early diverged lineages of winged insects (Pterygota), but its exact phylogenetic position remains uncertain (Prokop & Engel 2019). Kukalová-Peck (1983) suggested its placement as sister group to the Palaeoptera, comprising recent orders Ephemeroptera and Odonata. On the other hand, cladistic analysis carried out by Sroka et al. (2014) placed the group in sister position to Neoptera following the work of Willmann (1999).

According to some authors (e.g. Comstock 1918; Bolton 1921), the tracheae were deemed to determine the position of the main longitudinal veins, thus, the absence of developed veins in larval wing pads was not an obstacle to place immatures with well-preserved pattern of supposed tracheation in the system. According to the pretracheation theory, the tracheal pattern of the fossil wing pads could be homologized with the venational pattern of the adult wings. Although, the pretracheation hypothesis was rejected, and there can be great differences between the tracheation of the larval wing pads and the venation of adult wings, in some orders, e.g. in recent Ephemeroptera, Blattodea, Fulguroidea (Auchenorrhyncha) considerable homologies in the larval wing pad tracheation and adult wing venation are present (Fennah 1944; Landa 1948; Li et al. 2018).

To this day, fossil larvae of only the orders Palaeodictyoptera (Tab. S1) and Megasecoptera (Tab. S2) were described from the superorder Palaeodictyopterida. As the main difference between the larval wing pads of the two orders is deemed the formation of the costal area in the forewings. In palaeodictyopteran larvae the costal area usually shows an enlarged keel as a continuation of their prothoracic winglets. In the Megasecoptera the larval wing pads had only slightly broader costal area and the larvae did not possess the prothoracic winglets. However, in some specimens a long laterally protruding spines can be found, like in *Mischoptera douglassi* Carpenter & Richardson, 1968 (Megasecoptera: Mischopteridae) (Prokop et al. 2017a, b).

Goldenberg (1873) described a wing of a putative termite *Termes hageni* Goldenberg, 1873, which later Handlirsch (1906b) re-described as a larval wing pad of palaeodictyopteran insect of the genus *Palaeodictyopteron* Handlirsch, 1906, which he used for fossils that did not allow

further exact assignment within Palaeodictyoptera. In the same work, Handlirsch described three more palaeodictyopteran larval wing pads and one incomplete larval specimen of the genus *Palaeodictyopteron*. However, he did not specify any reasons, why the fossils should be assigned to Palaeodictyoptera. For the lack of supporting evidence were all these five taxa reconsidered by Carpenter (1948) and transferred to Insecta Incertae Sedis.

The first undoubtedly palaeodictyopteridan larval specimen bearing the outstretched wing pads (but the thorax is not preserved) from the Late Carboniferous of Mazon Creek, Illinois was described by Handlirsch (1911) as *Lameereites curvipennis* Handlirsch, 1911. Based on the shape and the pattern of supposed venation, these wing pads were assigned to the order Megasecoptera. According to Handlirsch, their outstretched position and overall appearance is an evidence, that the Megasecoptera could not be classified among holometabolous insects. Carpenter (1967) suggested the placement of *Lameereites* into the family Brodiidae, based on similarities with the larval wing pads of *Brodia priscotincta* Scudder, 1881, described by Bolton (1921). However, in the subsequent publication of Carpenter & Richardson (1968) stated, that the fossil is clearly related to representatives of the family Mischopteridae, but since it does not possess the same family traits, it was left unassigned to any family. After re-examination by Carpenter & Richardson (1968), the holotype of *L. curvipennis* showed wing pads composed of developing wings within wing sheaths and head with a beak and one preserved antenna. The haustellate mouthparts of larvae are similar to some adults of certain megasecopteran families, suggesting a similar diet of larvae and adults (Carpenter 1992). The wing pads have a well-developed corrugation and due to the presence of a sclerotized basal joint, it is possible that a muscle was attached at least to the base of the radius and the wing pad was thus partially movable (Prokop et al. 2017b). Haug et al. (2016) described another two fossils as *L. curvipennis* larvae, but since the pattern of wing pad tracheation was similar to that of the holotype of *Mischoptera douglassi* Carpenter & Richardson, 1968, these authors assumed, that *L. curvipennis* and *M. douglassi* were conspecific and suggested new name for the species, ?*Mischoptera curvipennis* (Handlirsch, 1911). However, Prokop et al. (2017b) based on the differences in commonly used characters in Megasecoptera taxonomy, supported the previous view of Carpenter & Richardson (1968) and restored the name *Lameereites curvipennis.*

Handlirsch (1919) described six larval wing pads from the Late Carboniferous of Coseley near Dudley, UK closely resembling representatives of *Brodia* Scudder, 1881, as several larval instars of *Brodia nympha* Handlirsh 1919, and he assigned the genus to the family Brodiidae within the order Megasecoptera. Bolton (1921) considered these and some additional specimens to be wings, and not wing pads, of the immature stages of *Brodia priscotincta* Scudder 1881,

and the whole family Brodiidae was assigned within the order Palaeodictyoptera based on large anal area and venational characters of the presumed wings. This was not generally accepted, and the family remained member of the Megasecoptera (e.g. Pruvost 1930; Carpenter 1967). Bolton (1921) stated, that the wings are too thin in contrast to thick wing pads of recent insects, still he agreed with Handlirsch, that the development of Palaeodictyopterida was gradual with progressive growth of wings and without the presence of resting or pupal stage.

Rolfe (1967) has re-examined a fossil of *Rochdalia parkeri* Woodward, 1913 which was previously described as Branchiopod (Woodward 1913), Trilobitomorpha (Stormer 1944), or Palaeanostraca (Hutchinson 1930). Based on the shape of pronotum with prothoracic lobes, wing pads with poorly developed "venation", and relatively large abdomen bearing annulated cerci, Rolfe re-described the fossil as an early instar larva of Palaeodictyoptera and together with another fossil, presumably later larval instar of *Rochdalia* having wing pads with well-developed "venation", suggested their placement in the family Breyeriidae. Rolfe (1967) also referred to an unidentified larva of cockroach with large oval process on the last abdominal segment with possibly branchial function.

Wootton (1972) re-described the holotype of *R. parkeri*. The specimen possesses probably hypognathous head, dorsal median keel extending for the whole length of thorax and abdomen, triangular prothoracal paranotal lobes with three straight "veins" and anterior peak, posterolaterally directed wing pads broadly fused with notum, mesothoracic wing pads with broad triangular costal area, metathoracic wing pads with narrower costal area, "venation" is poorly preserved, abdominal segments bear pointed posterolaterally oriented paranotal lobes and the last abdominal segment bears two wide cerci.

Wootton (1972) also described the second bigger *Rochdalia*-like specimen of Rolfe (1967) as a new species, *Idoptilus onisciformis* Wootton, 1972. On its thorax is visible dorsal medial keel, extending to fifth abdominal segment, prothorax bears paranotal lobes separated from the notum by furrow, the paranotal lobes bear one vein-like groove, proximally bifurcated, on meso-and metathorax are large posterolaterally oriented wing pads also separated from the thorax by wide trough. Both, fore and hind wing pads show quite detailed "venational" pattern and apparent corrugation, the costal area of fore wing is broad and triangular, while the costal area of hind wings is much narrower. At the bases of the wing pads are present elevated areas, which Wootton interpreted as possible precursors of articulation sclerites in composition similar to the pattern described in *Ostrava nigra* Kukalová, 1960 (Kukalová 1960). Each segment of the abdomen of *I. onisciformis* carries pointed, posterolaterally directed paranotal lobes.

Another fossil examined by Wootton (1972) was a larval wing pad and a part of thoracic notum, probably pronotum, (referred to as Nymph 3) found in the pelvic region of the holotype of tetrapod *Eugyrinus wildi* (Woodward, 1891) from the Late Carboniferous of Lower Coal Measures Formation, Lancashire, United Kingdom. The wing pad resembles the mesothoracic wing pads of *R. parkeri* and *I. onisciformis*.

The unidentified exuvia of the onisciform cockroach? larva of Rofle (1967) was re-examined by Prokop et al. (2018) and was attributed to Palaeodictyoptera, genus *Idoptilus*. The specimen shows small head surrounded by the prothoracic lobes, wing pads with pattern of light and dark transversal stripes and three posteriorly curved convex ridges corresponding to tracheal and lacunal patterns, mesothoracic wing pad with anterior keel, abdomen with laterotergites and last abdominal segment bearing a pair of triangular appendages and a single medial arched appendage. The authors agree with Rolfe's (1967) assumption, that the terminal abdominal appendages could have a branchial function. The appendages bear a prominent lateral lamella with dense pattern of fine tubercles, which indicates original presence of setae. Authors suggest that these appendages could represent tracheal gills similar to the tracheal gills of the damselfly larvae (Odonata: Zygoptera). This new interpretation points to the aquatic lifestyle for the onisciform larvae of *Rochdalia* and *Idoptilus*, as was previously suspected by Brongniart (1885, 1893) and Handlirsch (1906) and was further investigated in Prokop et al. (2019).

Carpenter & Richardson (1968) described fossil of immature megasecopteran from the Late Carboniferous of Mazon Creek, Illinois as *Mischoptera douglassi* Carpenter & Richardson, 1968 (Fig. 16c). This larva seemingly resembles the adults of *Mischoptera* Brongniart, 1894 (Megasecoptera: Mischopteridae) the most. The fossil shows probably hypognathous head with preserved antennae, presumed cross-section of the beak, prothorax with four long lateral spines on each side, meso- and metathorax with two anterior spines and one posterior spine to the wing pad on each side and numerous spines above and below the wing pads, wing pads basally oriented nearly perpendicular to the body, but then curved posterolaterally and abdomen with each abdominal segment bearing seven thick spines on its hind edge, similar to the spines of the adult *Mischoptera*, a pair of annulated cerci covered with setae emerging posteriorly from the tenth abdominal segment, and developing external genitalia. Authors explained, that in none of the recent insect orders do the larval wing pads develop in similar position to the body and that the position of the wing pads in *Lameereites curvipennis* would also be the same as in *M. douglassi*, if the thorax was preserved. Further examination of the wing pads showed the developing wings in a cuticular case with well-preserved longitudinal "veins" and only faintly discernible "cross-veins".

Another two fossil specimens of megasecopteran larvae were studied by Carpenter & Richardson (1968). The first one is almost complete larva, lacking only the last few abdominal segments. It was identified as a younger larval instar of *M. douglassi* (Fig. 16b), because of its smaller size and wing pads relatively smaller to the body size. According to the authors the wing pads are preserved in the same position as in the older larvae. The second larva was also identified as *M. douglassi* and lacks only the end of abdomen. Its body is about the same size and the wing pads are of the same size and position relatively to the body as in the *M. douglassi* holotype.
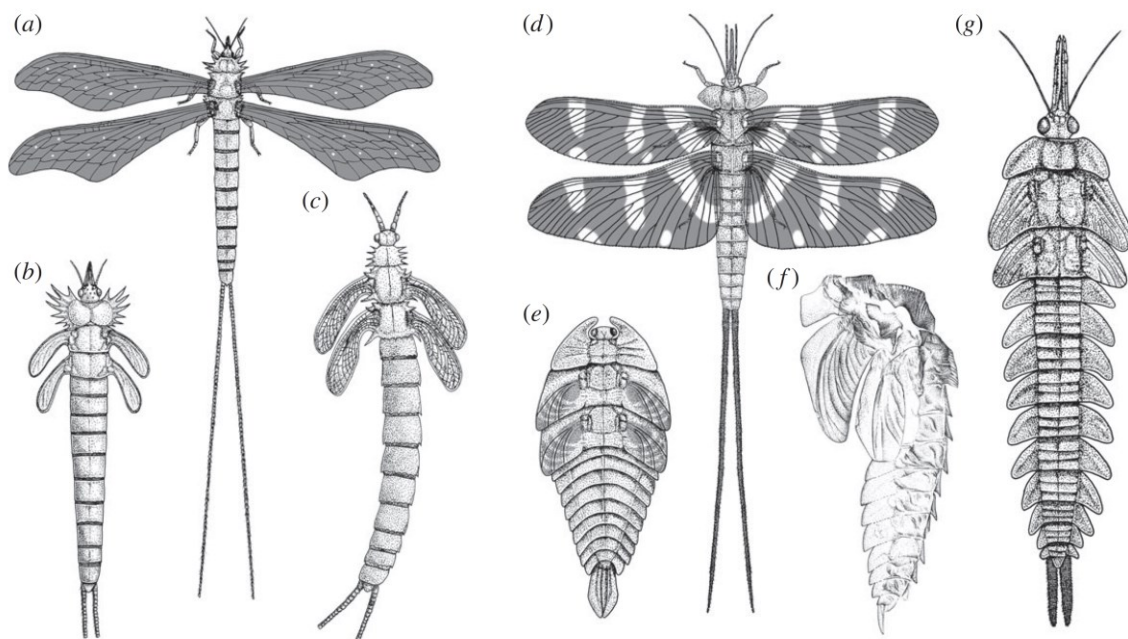

Figure 16: Disparity of morphotypes among larvae and adults of Late Carboniferous and Early Permian Megasecoptera (a-c) and Palaeodictyoptera (d-g) (Prokop et al. 2019). a, *Mischoptera nigra*, adult; b, *Mischoptera douglassi,* early larva; c, *Mischoptera douglassi*, older larva; d, *Dunbaria fasciipennis*, adult male; e, *Idoptilus* sp., larval exuvia; f, Palaeodictyoptera family indet., larval exuvia; g, *Bizarrea obscura*, larva.

Carpenter & Richardson (1968) concluded, that the posterolateral position of the wing pads relatively to the body was usual for the larvae of the family Mischopteridae and it was constant in the course of the larval ontogenetic development. This position of the wing pads was assumed to be common for all primitive palaeopterous insects, which was then supported by other published discoveries of another palaeodictyopteran larva (Carpenter & Richardson 1971) and also mayfly larvae from the Early Permian (Kukalová 1968) with the same wing pad position. Thus, the position of the wing pads lying longitudinally over the thorax and abdomen in recent

mayflies as well as dragonflies would be secondary adaptation for their aquatic lifestyle. Since the beak present in the larvae of Megasecoptera was fully developed, as in the adults of Megasecoptera and Palaeodictyoptera, Carpenter & Richardson (1968) assumed that the palaeodictyopteran larvae also possessed the beak. Both adults and larvae supposedly fed on the same food and because the adults were certainly terrestrial, the larvae also must have been terrestrial.

Based on the different body sizes, the three above mentioned specimens of *Mischoptera douglassi* larvae most likely represent at least two different instars. Prokop et al. (2017b) compared the two presumably older immature instars to known specimens of adults of *Mischoptera nigra* (Brongniart, 1885) and confirmed small increase in wing pad length and change to more outstretched position to the thorax.

The palaeodictyopteran larvae mentioned in Carpenter & Richardson (1968) was described as *Lycodemas adolescens* Carpenter & Richardson, 1971 from the Late Carboniferous and assigned to the family Lycocercidae. The fossil consisted of pterothorax with four wing pads and abdomen without tip. The wing pads showed faint pattern of "venation" and the wing sheath is clearly discernible along the developing wing. The narrow base of the wings pads is explained by the immature state of the wings. Numerous reticulate "cross-veins" are visible in some small areas of the wing pad. Carpenter & Richardson (1971) stated that the wing pads of *L. adolescens* and *M. douglassi* are connected to the body only in their articulation area. Another fossil of larval wing pad was mentioned in the study. This wing pad was three times the size of the *L. adolescens* wing pad and was described as older larva of *L. adolescens* or related species.

Three other representatives of megasecopteran immature instars were described by Kukalová-Peck (1975). The larval wing pad of *Cauloptera colorata* Kukalová-Peck, 1975, *Arcioneura juveniles* Kukalová-Peck, 1975 and *Alectoneura europaea* Kukalová-Peck 1975 all poses the supposed characteristic feature of the megasecopteran larval instar, the proximal bend of the wing pad, and because of their relatively bigger size and well sclerotized "venation", they should represent late or ultimate larval instars, or subimagos.

Kukalová-Peck & Peck (1976) described two wing pads of probable early instar larva of *Moravia convergens* Kukalová, 1964, representative of the family Calvertiellidae (Palaeodictyoptera). The wing pads show clearly visible developing wing inside the wing sheath, which are covered with "protruding tubercles" aligned in rows, each bearing one seta, supposedly functioning as a protection against abrasion. The wing pads have supposedly well-developed venation with discernible cross-veins and the same colouration pattern as adult wings. According to the authors, these wing pads are different from the wing pads of

contemporary winged insects by the fully developed longitudinal and transverse veins, corrugation, strong cuticle, developed articulation, which is supposedly indicated by their effortless separation from the thorax, and by their latero-posteriorly curved anterior and posterior margins. The authors assumed that the last characteristic was shared with other Palaeozoic members of Palaeoptera.

In the same study Kukalová-Peck & Peck (1976) described the fore wing of the supposed subimago of *Moravia convergens* Kukalová, 1964, which almost corresponded to the adult wing by its well-developed veins and dimensions, however, it supposedly differed from the adult by the latero-posteriorly curved margins of the wing and oblique position relatively to the body. The cuticle of the wings is thin and membranous, without tubercles found in the earlier instar. During ontogeny in *M. convergens* the wing pads should become gradually straightened with each moult and also, they gradually changed their orientation from longitudinal to perpendicular to the body. This was considered as one part of evidence for gradual development of Palaeozoic insects during ontogenesis, comparable to ametabolous development in apterygote insect groups.

Sharov & Sinitshenkova (1977) described fossil wing pad of *Goldenbergia fromosa* (Goldenberg, 1854) from the Early Permian as having almost the same pattern of "venation" as the adult wing and they assigned the genus to the family Dictyoneuridae (Palaeodictyoptera). In the same work was also described the specimen with wing pads of *Parathesoneura carpenteri* Sinitshenkova, 1977 (Palaeodictyoptera: Homoiopteridae). The hind wing pad is supposedly similar to the adult wing, only has less "veins" developed. The narrow base of the wing pads with distinct articulation sclerites and "venation" comparable to adult suggest, that the larva was capable of at least gliding flight.

Sinitshenkova (1979) studied the series of larval wing pads and subimaginal wings of *Tchirkovaea guttata* Zalessky, 1931, fragmentary early instar larva and hind wing pad of early instar larva of *Tchirkovaea sharovi* Sinitshenkova, 1979, and subimago, tips of subimaginal wings, a larva with elongated rostrum and ovipositor, early instar larva and one larval wing of *Paimbia fenestrata* Sinitshenkova, 1979, all specimens from the Late Carboniferous of localities Chunya and Paymbu in Russia. However, Sinisthenkova (1981) reasigned the *T. sharovi* into genus *Breyeria* de Borre, 1875 (Palaeodictyoptera: Beyeriidae) under new name, *Breyeria sharovi* (Sinitshenkova, 1979), based on the type of venation, strongly curved M and Cu branches and the presence of dense spots on the wing membrane assumed to represent hair bases.

Both genera, *Tchirkovaea* and *Paimbia*, were assigned to new family Tchirkovaeidae (Palaeodictyoptera). According to Sinitshenkova, this series of specimens supports the previous claims of Sharov (1973) and Kukalová (1969) that the development of the wings in Palaeodictyoptera was more gradual than in recent living insects, and that they had two subimaginal instars fully capable of flight. Even the earliest larval stages had developed venation, the wing pads enlarged progressively in the course of development, their bases become narrower and the axillary sclerites became visible. These wing pads of older larvae were probably movable. Also, the wing pads would change orientation through the ontogeny, from posterolateral to wing almost perpendicular to the body in subimago and perpendicular in imago, supporting the findings of Kukalová-Peck & Peck (1976). The larval wing pads and subimaginal wings show thicker membrane, presumably due to retention of the hypodermal layer needed for moulting. The cross-veins were always simple and very faintly visible in the larval stages, but in the imago, they were branched or form the true archedictyon. This type of metamorphosis was considered by Sinitshenkova (1979) as archimetaboly. The series of fossil wing pads and wings of *Tchirkovaea guttata*, as well as selected specimens of *Paimbia fenestrata* are re-examined in this thesis.

Another larva of the family Homoiopteridae (Palaeodictyoptera) was described by Kukalová-Peck & Richardson (1983) as *Adolarryia bairdi* Kukalová-Peck & Richardson, 1983. The larva was described as having "trilobite" type shape with thoracic terga, prothoracic "wings" strongly sclerotized, sculptured, granulated and covered with hairs, heart shaped prothoracic "wings" broadly attached prothorax with only narrow articulation area, the "wings" are strongly sclerotized and covered with hair, fore "wings" curved backwards, bent at about mid-wing, with articular sclerites compacted in a band at the base, and two middle abdominal segments bearing lateral lobes with oblique grooves, covered with hairs.

Ross (2010) re-examined the fossil of a palaeodictyopteran *Idoptilus peachii* (Woodward, 1887b) from the Late Carboniferous Middle Coal Measures, Greenhill Quarry, Ayrshire, Scotland, which was previously described as cockroach larva. The larva has onisciform body with small head, triangular paranotal lobes divided by groove from the prothorax, two pairs of wing pads with faint "venation", left wing pads showing corrugation, left hind wing pad with three colour bands, abdomen with medial keel and ten segments. The specimen looks similar to *R. parkeri* and *I. onisciformis* and based on the pattern of "venation" it is assigned to the genus *Idoptilus*.

Three hind wing pads from Late Carboniferous of Nord-Pas-De-Calais, France were described by Prokop et al. (2013) and putatively assigned to the representatives of the family Breyeriidae.

Due to different shapes and sizes of the wing pads and different angles with the thorax the authors assumed, that they represent different larval instars of one or more species. Two wing pads had well preserved corrugation and discernible partially developed articulation. All wing pads had a wide cubito-anal area and were regarded hind. When compared with the wing pads of *R. parkeri* and *I. onisciformis* the wing pads have similar pattern of "venation", but were probably of older instar, because they are more outstreched and have discernible articulation.

Prokop et al. (2016) described a larva from the Late Carboniferous of Mazon Creek, Illinois, USA as *Bizzarea obscura* Prokop et al., 2016 (Fig. 16g) and assigned it to family Spilapteridae (Palaeodictyoptera). The larva has poorly preserved head with partly preserved rostrum and long filiform antennae, prothoracic lobes without preserved tracheation, small meso- and metathoracic wing pads with partly preserved venation, corrugated, and oriented obliquely backwards, metathoracic wing pads slightly shorter than mesothoracic and basally broader, slender abdomen with ten segments bearing posteriorly pointed laterotergites, ovipositor beneath the seventh to tenth abdominal segments and the last abdominal segment with two stout annulated cerci covered with setae.

Haug et al. (2016) tentatively attributed fossil of isolated wing pad from as *?Mischoptera curvipennis* (Megasecoptera). This was reconsidered after the re-examination of the fossil by Prokop et al. (2017a). Since the mesothoracic wing pad bears a very broad costal area with keel, it was placed in the Palaeodictyoptera. The specimen also shows developing articulation of the wing pads with distinctive articulation sclerites and broad posterior connection of the wing pad with notum, similar to the condition in *R. parkeri* and *I. onisciformis*. The wing pad tracheation pattern is also very similar to that of *I. onisciformis*, therefore the wing pad could belong to a representative of the same or some related genus.

Prokop et al. (2017a) studied an incomplete larval exuvia from the Late Carboniferous of Upper Silesian Coal Basin, Poland, with triangular mesothoracic wing pad sheath and preserved corrugated pattern of tracheation or vein lacunae. Both wing pads had prominent joint at the base, but the prothoracic lobe was only weakly jointed with the prothorax suggesting only limited mobility.

Just recently Sinitshenkova (2020) assigned an isolated hind wing pad from the Early Permian of Vorkuta in Russia to the species *Vorkutoneura variabilis* Sinitshenkova 1977 (Palaeodictyoptera: Spilapteridae). The wing pad has an expanded anal region and the pattern of "venation" is similar to that of the adult wing.

1.5.1. The views on the course of ontogeny in the Palaeodictyopterida

In recent hemimetabolous insects, the wing pads remain relatively small during the ontogenetic development. During the final larval instar, the developing adult wings are crammed within the wing sheaths and after the final moult the difference in size between the larval wing pads and the adult wings is very significant (e.g. Holdsworth 1942). The fundamental changes in the transformation from larval to adult stage are accumulated into this final so-called metamorphic stage (Kukalová-Peck 1991). For example, the venation in the recent insects (except for Ephemeroptera, where the stage of subimago is still present) arise only in this time with change to imago. In neometabolous orders the metamorphic stage is represented by one or more resting stages, while in the Holometabola with complete metamorphosis, the metamorphic stage is the pupa and it is only at this stage that the outer wing buds are formed (Sehnal et al. 1996).

The postembryonic development in Palaeodictyoptera was considered gradual by several authors and may have involved up to several subimaginal instars or even moulting of adults (as in recent ametabolous Archaeognatha and Zygentoma), and the growth of the wings should also be gradual with steady straightening of the curved wing pads and continuous change of their position relative to the body from longitudinal to perpendicular (Carpenter & Richardson 1968; Sharov 1973; Kukalová-Peck 1976, 1978, 1983). Kukalová-Peck (1978) in her work on the origin and evolution of wings stated, that the larval wing pads of all Palaeozoic ancestral insects were articulated with tergum, had developed venation and were fully functional as wings. She considered this one of the proofs of the exite origin hypothesis.

In contrast to these hypotheses, Prokop et al. (2017a) showed, after revision of palaeodictyopteran larvae, that the pronotal lobes and wing pads of *R. parkeri* and *I. onisciformis* were indeed articulated, but anteriorly and particularly posteriorly were broadly fused with notum. The wing pads also show apparent corrugation, the longitudinal ridges, which presumably represents the pattern of wing pad tracheation and developing wing veins, correspond with the later longitudinal sectors. After re-examination of some larvae of Palaeodictyoptera, Prokop et al. (2016) contradicted the hypothesis of gradual development and wing growth in at least some groups and representatives within the Palaeodictyoptera. For example, the larva of *Paimbia fenestrata* of the family Tchirkovaeidae carried the wing pads pointed backwards along the body, but a well-developed endophytic ovipositor and body length approaching the length of adult suggested, that it was an older larva or even subimago rather than an early instar. Similarly, the larva of the spilapterid genus *Bizarrea* possessed wing pads relatively short in proportion to its large body, implying that they were early instar larvae, but at the same time their bodies are larger than all the adult bodies of members of the family so far

described. On the contrary, several larvae within the Breyeriidae family with small wing pads are rather representatives of early instar larvae rather than subimagos, although their wing pads lie almost perpendicular to the body axis. Therefore, Prokop et al. (2016) supposed, that the development of some representatives of the Palaeodictyoptera could more closely resembled the development of recent palaeopterous insects, such as Odonata or Ephemeroptera, but also other hemimetabolous orders of Neoptera, such as Plecoptera or Orthoptera.

2. Material and methods

2.1. Terminology of wing venation and tracheation

This thesis follows the wing venation nomenclature of Riek and Kukalová-Peck (1984), Kukalová-Peck (1991), modified according to the venation scheme of Snodgrass (1935), supported by the recent works (Li et al. 2018; Schubnel 2020). The following abbreviations for the wing veins are used: C, costa; CA, costa anterior; CP, costa posterior; ScA, subcosta anterior; ScP, subcosta posterior; R, radius; RA, radius anterior; RP, radius posterior; M, media; MA, media anterior; MP, media posterior; Cu, cubitus; CuA, cubitus anterior; CuP, cubitus posterior; PCu, postcubitus; A, anal vein; 1A, first anal vein.

The lacunae of the developing wing pads are named according to the veins they precede and the tracheae of the developing wing pads are named according to the lacunae, they pass through. Since the developing venation in the larval wing pads is in the form of lacunae, through which the tracheae pass, it is not certain, which of these structures are clearly discernible in the fossil larval wing pad. In the systematic parts describing the fossil specimens, the developing pattern of venation thus can be interpreted as consisting of vein precursors (i.e., lacunae, or tracheae).

2.2. Fossil material

2.2.1. Specimens origin and location

All fossil specimen re-examined in this thesis are stored in the collection of Paleontological Institute of Russian Academy of Sciences, Moscow, Russia (abbreviation PIN).

The prefixes in PIN:

Series 2293 – Paymbu River locality, Tunguska Basin, Krasnoyarsk Territory, Russia; Late Carboniferous, Kasimovian, Kata Formation.

Series 2854 – Kuray, Altai Republic, Russia.

Series 2992 – Paymbu River locality, Tunguska Basin, Krasnoyarsk Territory, Russia; Late Carboniferous, Kasimovian, Kata Formation.

Series 3115 – Chunya River locality, Tunguska Basin, Krasnoyarsk Territory, Russia; Late Carboniferous, Kasimovian, Kata Formation.

List of examined specimens:

Undescribed specimens: 2854/2, 5384/79

Palaeodictyoptera: Homoiopteridae
Parathesoneura carpenteri Sinitshenkova, 1977: No. 3115/26

Palaeodictyoptera: Tchirkovaeidae
Tchirkovaea guttata Zalessky, 1931: Nos. 3115/29; 3115/63, 3115/68, 3115/152, 3115/155, 3115/160 and 3115/167
Paimbia fenestrata Sinitshenkova, 1979: Nos. 2293/4 (holotype), 2293/6, 2293/7, 2992/1
Paimbia ultima Sinitshenkova, 1979: No. 3115/192 (holotype)

2.2.2. Methods of observation

The fossil material re-examined in this thesis is mainly the unique series of fossik wing pads fossils of different developmental stages attributed to *Tchirkovaea guttata* Sinitshenkova, 1979. Seven specimens were selected (3115/29; 3115/63, 3115/68, 3115/152, 3115/155, 3115/160, 3115/167) based on the quality of its preservation for further re-examination, and other specimens which could not be examined (unavailable in the collection) are re-described based on the drawings and photographs in the study of Sinitshenkova (1979). Furthermore, the following material was examined for comparative reasons: one wing pad, one partially preserved nymph, one partially preserved subimago and one adult wing of *Paimbia fenestrata* Sinitshenkova, 1979 (holotype 2293/4, paratypes 2293/6, 2293/7, 2992/1), one fragment of imaginal wing of *Paimbia ultima* Sinitshenkova, 1981 (holotype 3115/192), one fragmentary wing pad *Parathesoneura carpenteri* Sinitshenkova, 1979 (3115/26), and one undescribed larval wing pad fossils (2854/2).

Photographs of the specimens were taken with a Canon D550 digital camera with MP-E 65 mm and EF 50 mm macro-lenses in a dry state. Selected specimens were further observed using Leica MZ12.5 stereomicroscope in a dry state and line drawings were composed using a stereomicroscope with camera lucida attachment. Photographs were processed in the editing software Adobe Photoshop CS8 and some by the focus-stacking software Zerene Stacker.

The micrographs of the fragment of imaginal wing of *P. ultima* were obtained using an environmental scanning electron microscope Hitachi S-3700N at the Department of Paleontology, National Museum in Prague.

2.3. Recent material

The representatives of recent mayflies (Ephemeroptera) with the subimaginal instar present in their development (Ephemeroptera) were collected in the time range from May to December 2019, with a net in the ponds behind the building of the Faculty of Science, Viničná 7, Praha 2, Czech Republic. The examined species was identified as *Cloeon dipterum* (Linnaeus, 1761) (Ephemeroptera: Baetidae) (n = 27).

These specimens were observed alive in water or glycerol on a slide and covered by the cover slide. Bigger specimens were killed by exposure to ethyl acetate and the wing pads were carefully removed from the body, put in a drop of water on a slide and covered by the cover slide. Microphotographs of these specimens were taken in the Olympus SZX10 microscope and focus stacked in focus stacking software, Photoshop CS 8, and Zerene Stacker.

Last larval instars were kept in captivity and left to moult into subimago. One subimago was than left overnight in a plastic bottle to moult into a imago. The wings pads of female last larval instar and subimago were also measured.

To roughly sort the larval specimens based on the progress of development, the heads of the specimens were measured in their widest point and the samples were placed in an order according to the measurements.

3. Results

3.1. Fossil material - Systematic part

Superorder Palaeodictyopterida

Order Palaeodictyoptera Goldenberg, 1877

Family Tchirkovaeidae Sinitshenkova, 1979

Genus *Tchirkovaea* Zalessky, 1931

Type species. *Tchirkovaea guttata* Zalessky, 1931

Tchirkovaea guttata Zalessky, 1931

Zalessky (1931: p. 403, fig. 4): Megasecoptera: Diaphanopteridae

Rohdendorf (1961: p. 78-79, fig. 31): Megasecoptera: Diaphanopteridae

Sinitshenkova (1979: p. 192, figs. 1, 2, 3, 4, 5, plate VII 1-7): Palaeodictyoptera: Tchirkovaeidae

Holotype: stored in the collection of M. D. Zalessky (Vernadsky state geol. Mus., Moscow).

Holotype location: North Oungua river, Tcheremitchkino, Kuznetsk Basin; Late Carboniferous

Re-examined material:

PIN RAS Moscow collection: 3115/29; 3115/63, 3115/68, 3115/152, 3115/155, 3115/160, 3115/167; 2854/2; 5384/79

Described based on the figures in Sinitshenkova (1979): 3115/53, 3115/66, 3115/151, 3115/153, 3115/157 3115/191, 3115/193

Larval wing pads

Undescribed Specimen no. 2854-2 (Fig. 17; Fig. S1A) – part and counterpart

Description. Well preserved part and counterpart of larval wing pad, creased; preserved part of notum, detached from the wing pad; outer margin considerably enlarged; wing pad of triangular shape, anterior margin curved, posterior margin rather straight, proximally convex; costal area rather broad; cross-vein precursors not discernible; main vein precursors corrugation apparent; ScP concave, straight, reaching RA at 3/4 of wing pad length (about 6 mm from wing pad base); RA and RP running together, diverging at about 3 mm form wing base; RA simple, convex, curved towards the wing apex; RP concave, pectinated, ending with three branches on the posterior margin; first branch of RP diverged at 2/3 of the wing pad length at about half the RP length; stem M runs along RA/RP, then slightly diverging posteriorly, MP diverges from MA at 1/3 of the wing pad length (about 2.8 mm from base), just before point of RA/RP divergence; MA convex, MP concave, both vein precursors simple, ending on the posterior margin; Cu stem runs further from M, divided into CuA and CuP at 1/5 of wing pad length (1.6 mm form base); CuA convex, CuP concave, both vein precursors simple, ending on the posterior margin; anal area rather broad with four simple vein precursors.

Dimensions. Total length of fossil about 10 mm, wing pad length from base about 8 mm, maximum width about 5 mm.

Remarks. The surface of the wing pad is considerably creased, making it harder to distinguish the developing pattern of venation, and the wing pad outer margin is significantly enlarged. Both features confirm the presence of a developing wing within cuticular case.

The wing pad belonges to the representative of the Palaeodictyoptera group based on the presence of precursors of all main longitudinal veins with distinct corrugation (Carpenter 1992). No developing cross-veins are discernible, the pattern of developing venation is comparable with the pattern of adults of *T. guttata*. The ScP precursor is simple and enters R distally (Sinitshenkova 1979).

In some families of Palaeodictyoptera, the fore and hind wings had similar form (e.g., Dictyoneuridae, Lycocercidae), in others the hind wings were much broader (e.g., Calvertiellidae, Breyeriidae, Homoiopteridae, Lithomanteidae, Spilapteridae), in some families the hind wings are considerably smaller or even completely absent (e.g., Eugereonidae, Megaptilidae, Diathemidae, Permothemistidae). Another difference could have been the narrower costal area of hind wing (e.g., Dictyoneuridae, Mecynosotmatidae). In the family

Tchirkovaeidae, the hind wings were wider than the fore wings, which was the case also for *Tchirkovaea guttata*. The hind wings had narrower costal area, rather straight anterior margin, broader anal area and more convex posterior margin proximally, generally having rather triangular shape, while the fore wings were narrower with broad costal area and had more oval shape (Sinitshenkova 1979). This wing pad shows a relatively broader costal area with considerably convex anterior margin, and therefore it is considered a fore wing pad. The wing pad is rather short, but it would probably elongate and become narrower progressively through the subsequent development.

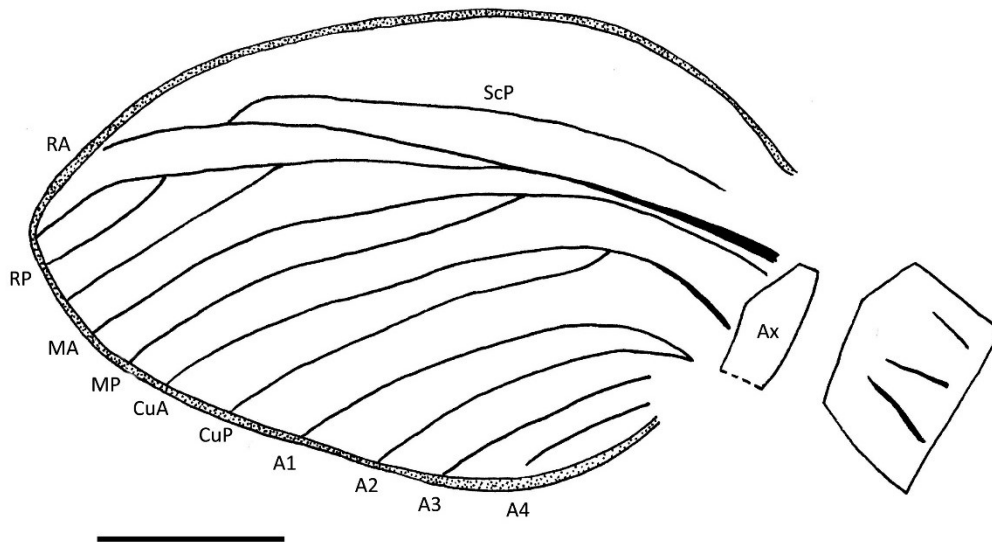A single axillary plate is distinguishable near the bases of RA/RP, M and CuA/CuP.

Figure 17: *Tchirkovaea guttata*, line drawing of the specimen 2854/2. A1-A4, anal veins; Ax, axillary plate; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; RA, radius anterior; RP, radius posterior; ScP, subcosta posterior. Scale bar = 2 mm.

Specimen no. 3115/29 (Fig. 18; Fig. S1B)

Sinitshenkova 1979, p. 198, fig. 4a; plate VII, fig. 1

Description. Larval wing pad well separated from thorax, with markedly enlarged outer margin; wing pad of rather oval shape, anterior margin curved; wing pad rather thick, darkly coloured, with numerous circular points or thickening of the membrane; costal area rather broad bearing numerous oblique cross-vein precursors in the proximal 2/3 of the wing pad; main longitudinal vein precursors distinctly corrugated; numerous cross-vein precursors present between the RP, M, Cu and A; ScA faintly distinguishable, running from the upper base of ScP to reach the

costal margin in the length of about 2.2 mm from the base of ScP; concave ScP simple, running parallel to R, reaching RA at about 4/5 of the wing pad length; RA and RP run together from wing pad base, diverging at about 1/3 wing pad length from base; RA convex, slightly curved near wing pad apex, simple, ending at the wing pad apex; RP concave, branched distally, ending with three branches on posterior wing pad margin; first branch of RP diverged from RA at about 4/5 wing pad length, behind the mid point of the RP; stem M runs close to R, slightly curved, diverging into MA and MP further from wing pad base than the divergence of RA and RP; both MA and MP slightly curved distally; MA convex, simple; MP concave, ending with terminal twig reaching the posterior margin; Cu stem runs more distant from M stem; CuA and CuP diverge at about 3.5 mm from the wing base; CuA convex, simple; CuP concave, simple; PCu developed, joins CuP shortly after its divergence from CuA; anal area rather broad with six anal branches, slightly curved.

Dimensions. The length of the wing pad about 14.8 mm, maximum width about 5.9 mm.

Remarks. The surface of the wing pad is wrinkled in some areas and the wing pad outer margin is markedly thick. Both features confirm the presence of a developing wing within cuticular case. The wing pad is rather narrow of relatively oval shape, its costal area is broad and anterior margin convex, and based on the comparison with other available wing pads and imaginal wings of the species *T. guttata* it is considered as a fore wing pad.

Sinitshenkova (1979) interpreted this specimen as for wing pad belonging to the early instar larvae, probably as it is the smallest one in the fossil series. Since the wing pad is 1.5x longer than the previously undescribed wing pad (2854/2) it could represent one of the subsequent early instars.

Sinitshenkova (1979) described the fore wing pads of *T. guttata* larvae as having five anal vein precursors in the anal area, but on her figure she distinguished seven anal veins, in reality, there are six distinguishable anal vein precursors on this wing pad. The author also figured two cross-veins between the M stem and CuA, one basally, the second one just behind the point of branching of MA and MP. But after closer observation, these are probably only wrinkles on the cuticle membrane of the wing pad.

This fore wing pad differs from the previous one (specimen 2854/2) in the order of points of divergence of RA/RP and MA/MP and also in more distally branched vein precursor of MA.

There are nygma-like structures present in many cells with 0.37 in diameter on average (Fig. S3A). On the present fore wing pad of *T. guttata* early larva there are at least twenty-one similar structures, some are better preserved while others not. The wing base is not clearly preserved.
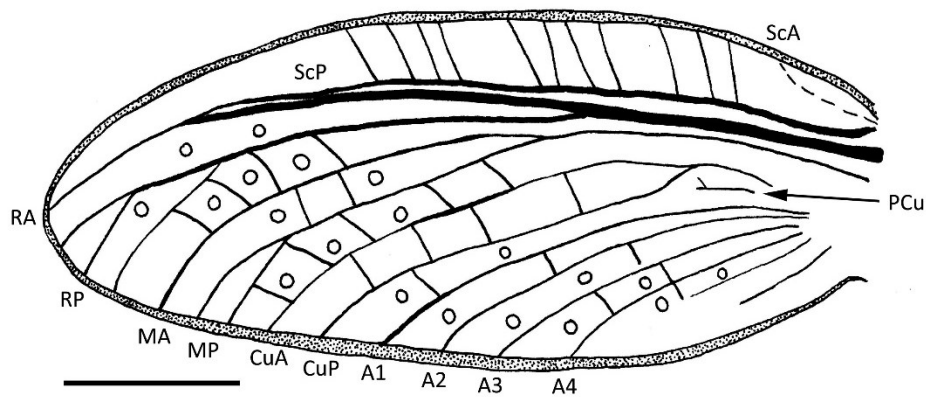
Figure 18: *Tchirkovaea guttata*, line drawing of the specimen 3115/29. A1-A4, anal veins; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; PCu, postcubitus; RA, radius anterior; RP, radius posterior; ScA, subcosta anterior; ScP, subcosta posterior. Scale bar = 3 mm.

Specimen no. 3115/160 (Fig. 19; Fig. S1C)

Sinitshenkova 1979, p. 196

Description. Nearly complete larval wing pad, enlarged outer margin; posterior notal fusion and part of notum preserved; thick, darkly coloured, triangular wing pad, anterior margin convex distally, posterior margin straight, then more convex proximally; all main longitudinal vein precursors corrugated; costal area narrow, with few cross-vein precursors; one cross-vein precursor preserved between M and CuA; ScA running from the upper base of ScP, reaching the costal margin at about 3 mm from the wing pad base; ScP concave, simple, straight, running parallel to R, reaching RA at about the 3/4 wing pad length; RP diverging from RA at about 1/3 of the wing pad length; RA convex, slightly undulating, simple; concave RP forked distally at about 2/3 of the wing pad length; stem M runs closely to R, divided into MA and MP just before the point of RA/RP divergence; convex MA simple, curved towards the posterior margin of the wing pad; MP concave, forked distally, ending with two branches on the posterior margin; stem Cu runs more distant from M, CuP diverging at about 1/5 of the wing pad length (2.6 mm from the wing pad base); CuA convex, CuP concave, both distally forked ending with two branches on posterior margin; PCu discernible, adhered to CuP shortly after its divergence; anal area broad with six preserved branches, all simple.

50

Dimensions. Total fossil length 15.5 mm, wing pad length about 13.8 mm, maximum wing pad width about 6.7 mm.

Remarks. This wing pad is just slightly shorter than the previous one (specimen 3115/29), with the similar appearance of the wing pad membrane. Anterior part of wing pad tip is not preserved. The markedly thickened outer margin is visible, with the vein precursors well distinguishable and the wing pad surface is wrinkled in some places, which confirms the presence of developing wing within cuticular case.

It was probably hind wing pad based on the overall triangular shape of the wing pad, its rather narrow costa area, broad anal area with six anal vein precursors and straight distal part of the posterior wing pad margin.

When compared with the previous fore wing pad (specimen 3115/29) the wing pad is only slightly shorter (1 mm), therefore it could be a wing pad of the same or just previous instar. Both wing pads have the vein precursor of ScA present. The differences from the fore wing pad (specimen 3115/29) are in CuA and CuP both ending with two branches on the posterior margin and in the position of the point of divergence of RA and RP, which is just a little further from the wing base than the point of branching of MA and MP, similarly to the situation in the smallest fore wing pad (2854/2).

In contrast to the younger larval fore wing pad (2854/2) this wing pad (3115/160) is elongated and relatively narrower, the costal area is also narrow, the veins MP, CuA and CP are all distally branched.

Slight undulation of the costal margin is present probably due to preservation. This relatively well-preserved wing pad is not figured in the original paper of Sinitshenkova. It is described in the text as the only impression of the "hind wing of an early instar nymph on which three vein precursors (MP, CuA, and A) have short terminal forks". Posterior notal fusion of the wing pad is preserved.
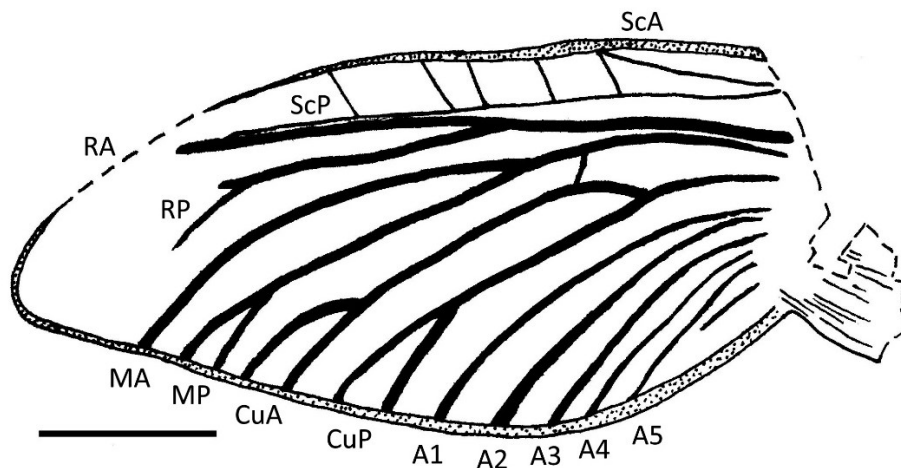
Figure 19: *Tchirkovaea guttata*, line drawing of the specimen 3115/160. A1-A5, anal veins; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; RA, radius anterior; RP, radius posterior; ScA, subcosta anterior; ScP, subcosta posterior. Scale bar = 3 mm.

Specimen no. 3115/157

Sinitshenkova, 1979, p. 199, fig. 5a; plate VII, fig. 2

Description. Complete larval wing pad connected to part of notum; triangular wing pad, posterior margin rather straight distally, proximally convex; costal area narrow, with numerous cross-vein precursors; numerous cross-veins present, especially in the area between radius and analis; ScP runs parallel to R, reaching it at about 3/4 of wing pad length (about 11.4 mm from R stem base); RA/RP straight, RP diverging from RA at about 1/3 wing pad length (5 mm from RA/RP base); RA simple, curved towards the wing pad apex, ending before the apex; RP pectinated, ending with three branches on posterior margin of the wing pad; first RP bifurcation at about half the RP length; M stem running closely to RA/RP, than diverging posteriorly, dividing into MA and MP closely after the point of divergence of RA and RP, closer to mid wing pad; MA and MP simple, curved towards and ending at the posterior margin; stem Cu runs more distant from M, curved posteriorly, CuP diverging from CuA at about 1/5 of the wing pad length; CuA and CuP simple, ending on the posterior margin; PCu not distinguished; anal area broad with up to seven simple branches.

Dimensions. Full length of the fossil specimen about 18 mm; length of the wing pad from the distal edge of axillary area to tip about 14.7 mm, maximum width about 7 mm.

Remarks. The specimen could not be found in the PIN RAS collection. The description is thus provided based on the figure in Sinitshenkova (1979). The wing pad is presumably hind, based on its overall shape, the narrow costal area and broad anal area. Sinitshenkova regarded this specimen as an early instar hind wing pad.

It is about 1 mm longer than the previous hind wing pad (specimen 3115/160) and about the same length as the fore wing pad (specimen 3115/29). Therefore, it could represent either the same instar as specimen 3115/29 or just the subsequent larval instar. The wing pad differs form the previous hind wing pad (3115/160) in the position of the RA/RP divergence point, which lies more proximally than the point of branching of MA and MP, same as in the fore wing pad (3115/29). In contrast to the hind wing pad specimen 3115/160 the vein precursors of MP, CuA and CuP are simple (as in specimen 2854/2).

Specimen no. 3115/155 (Fig. 20; Fig. S1D)

Sinitshenkova 1979, p. 199, fig. 5b; plate VII, fig. 3

Description. Nearly complete larval wing pad with enlarged outer margin, with dark coloration; triangular shape of wing pad, anterior margin nearly straight, convex distally, posterior margin curved in the proximal part with broad anal area; costal area relatively narrow; main longitudinal vein precursors corrugated; numerous cross-vein precursors present in the M and Cu area; concave ScP simple, straight, runs parallel to R, reaching RA at about 3/4 of wing pad length; RA and RP separate at about 1/3 wing pad length; RA convex, simple, curved towards the wing pad apex, ending before the apex; RP concave, pectinated distally and ending with four branches on the posterior margin; first RP branch diverging at about 2/3 wing pad length, at about half of the length of RP; stem M not discernible; MA proximally attached to RA/RP, diverging at about mid wing pad, simple, convex, curved towards the posterior wing pad margin; concave MP running close to R at the base, then strongly diverged posteriorly, ending shortly forked with two branches on the posterior margin; base of Cu stem not preserved, distal part of stem of Cu remote from M stem, CuP diverged from CuA at about 1/4 of the wing pad length; CuA convex, simple; CuP concave, simple; anal area rather broad with seven simple branches.

Dimensions. Length of the wing pad about 19 mm, maximum width about 10.6 mm.

Remarks. The wing pad has a missing basal part and the preservation is poor. The wing pad surface is creased, which makes it more difficult to interpret the partially distorted pattern of developing wing venation and corrugation, the MA divergence from RP could possibly resulted

from this. The thick outer margin of the wing pad is discernible. The creasing is probably caused by the cuticular case crumpling. The cross-vein precursors are hardly discernible from the wrinkles.

Sinitshenkova (1979) described this wing pad as hind wing pad of middle instar larva. The shape of the wing pad is rather triangular, with narrow costal area, broad anal area, and straight distal part of the posterior wing margin.Therefore it probably represents the hind wing pad. It is about 1.25x longer than the previous hind wing pad (specimen 3115/157), and it could represent an older larval instar.

In contrast to the previous larval hind wing pads (specimens 3115/160, 3115/157) the MA appears to be fused with RP for a considerable distance, the common basal trunk of MA and MP is not preserved.
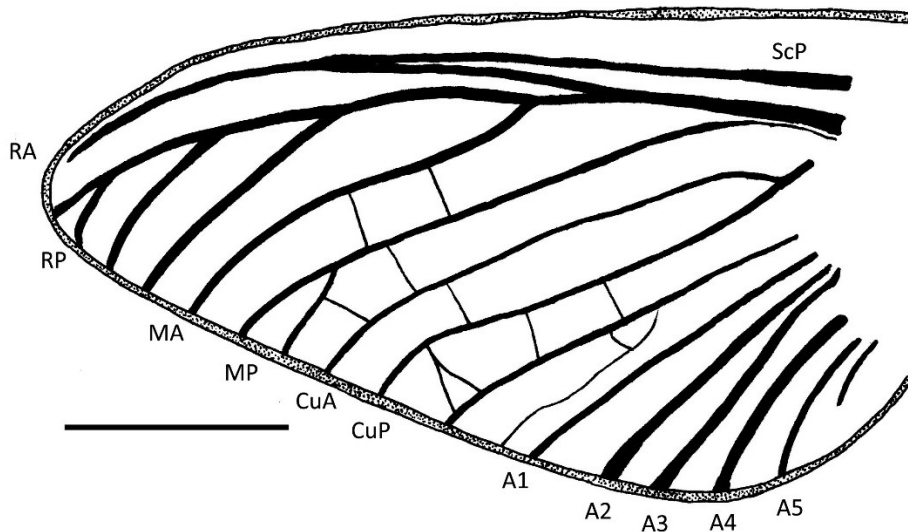

Figure 20: *Tchirkovaea guttata*, line drawing of the specimen 3115/155. A1-A5, anal veins; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; RA, radius anterior; RP, radius posterior; ScP, subcosta posterior. Scale bar = 5 mm.

Specimen no. 3115/167 (Fig. 21; Fig. S1E)

Description. Poorly preserved wing pad, creased; enlarged outer margin discernible; triangular shape of wing pad; anterior margin rather straight, posterior margin strongly curved in the proximal part; costal area relatively narrow; concave ScP running parallel to R, nearly straight; division of RA and RP in about 1/3 of wing pad length; RA convex, slightly undulated, simple;

RP concave, straight, distally pectinated ending with 3 branches, first branch diverging at about 2/3 of the wing pad length, at about half the RP length; stem M running parallel to stem R at the wing pad base, than curved posteriorly; M divided to MP and MA in the same distance from wing pad base as the point of divergence of RP from RA; MA simple, convex, slightly curved towards the posterior margin; MP simple, concave; Cu stem running more distanced from M stem, forked into CuA and CuP at about 4 mm from the wing base; CuA convex, anteriorly pectinated with 3 branches; CuP simple, concave; anal area broad with eight branches; first and seventh anal vein precursors forked.

Dimensions. Wing pad length about 21 mm, maximum width 11.3 mm.

Remarks. The wing pad is poorly preserved with some parts peeled off. The surface of the wing pad is creased, making the recognition of the main longitudinal vein precursors difficult and the corrugation is partially distorted. The most distal parts of vein precursors are not preserved and no cross-vein precursors discernible. Considering the shape of the wing pad, the narrow costal area, and the broad anal area it is probably a hind wing pad. This specimen was not mentioned in the original work of Sinitshenkova (1979).

The wing pad is about 2 mm longer than the previous hind wing pad (specimen 3115/155) and could have belonged to the same or older instar larva. The differences to the previous hind wing pad is the only three branched RP, the M stem normally developed, with MA and MP diverging at about the same length from base as is the point of divergence of RA and RP, the simple MP, the three branched CuA, and the branched first anal vein precursor.
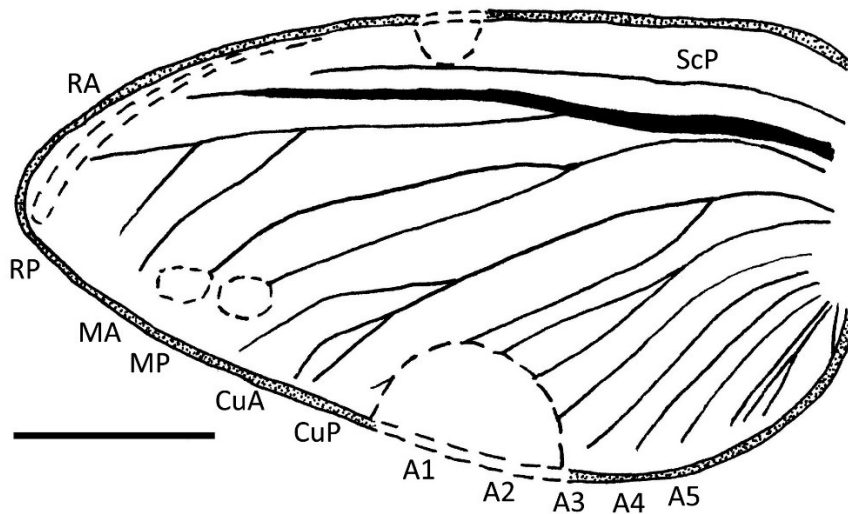
Figure 21: *Tchirkovaea guttata*, line drawing of the specimen 3115/167. A1-A5, anal veins; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; RA, radius anterior; RP, radius posterior; ScP, subcosta posterior. Scale bar = 5 mm.

Specimen no. 3115/153 (Fig. S1F)

Sinitshenkova 1979, p. 198, fig. 4b; plate VII, fig. 4

Description. Fragmentary larval wing pad, darkly pigmented with light circular spots located in the middle of the venation cells (eventually the membrane of circular spots missing due to taphonomy); wing rather oval; costal area broad proximally, with numerous cross-vein precursors; many cross-vein precursors present in the RP – A region; ScP straight, running parallel to RA; RP diverging from RA at about basal third, both vein precursors straight; M stem running close to RA/RP, than diverged posteriorly, MP diverging from MA about 4 mm after the point of divergence of RA and RP; MA simple, slightly curved distally, ending on the posterior margin; MP pectinated, ending with three posterior branches; Cu stem basally remote from M stem, CuA and CuP diverged proximally; CuA ending with short fork on the posterior margin; CuP simple, slightly undulated, ending on the posterior margin; anal area with six branched preserved, first anal vein precursor forked distally.

Dimensions. Length of incomplete wing pad about 45.2 mm, full wing pad length estimated on about 50 mm, maximum width about 24 mm.

Remarks. The wing pad is missing basal and apical parts, distal parts of ScP, RA and RP are

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not preserved. The wing pad is darkly coloured with light spots in the middle of the membrane cells. Sinitshenkova (1979) observed, that this pattern can be found also in the specimen 3115/53 (p. 194), however this cannot be confirmed, since the specimen was not available for re-examination. This pattern of the wing colouration is preserved in the presumed subimaginal specimens (3115/ 151, 3115/152, 3115/63).

The costal area of the wing pad is rather broad proximally and the wing pad seems rather oval in shape, thus, it probably represents fore wing pad.

According to Sinitshenkova (1979) it was the fossil fore wing pad of a later instar larva; its length is only about 2 mm less than the length of the following hind wing pad (specimen 3115/53).

When compared to the fore wing pad of an early larva (specimen 3115/29), the MP ends with three branches, not two, the cross-vein precursors are well developed. The relative position of the points of divergence of RA/RP and MA/MP is at the same level.

Specimen no. 3115/53

Sinitshenkova 1979, p. 199, fig. 5c

Description. Larval wing pad; wing pad triangular, anterior margin rather straight, posterior margin distally straight, convex proximally; costal area narrow with many cross-vein precursors; numerous cross-vein precursors in R-A area; ScP straight, simple, running parallel to R, reaching RA at about 3/4 of wing pad length; Radius slightly undulated, RP diverging from RA at about 1/3 of wing pad length; RA simple, straight, distally curved, ending at the wing pad apex; RP pectinated, with three branches ending on the posterior wing pad margin; first branch of RP diverging at about 2/3 of estimated wing pad length, at about half the length of RP; M stem running closely to R, then diverged posteriorly, divided into MA and MP just behind the point of RA and RP divergence; MA simple, slightly curved towards the posterior margin; MP ending with short fork at the posterior margin; Cu diverged posteriorly from M stem, CuA and CuP separate at about 1/5 of the wing pad length; CuA simple undulated, ending on the posterior margin; CuP forked, ending with two branches on the posterior margin; anal area broad with six preserved branches and proximal part missing.

Dimensions. Length of the incomplete wing pad about 48.1 mm, estimated wing pad length 52 mm, maximal width about 26 mm.

Remarks. This specimen could not be re-examined, and the description is provided based on the figure of Sinitshenkova (1979). The shape of the wing seems rather triangular, with narrow

costal area and broad anal area Therefore, it is probably a hind wing pad. The basal part of the wing pad is not preserved.

Sinitshenkova (1979) described the wing pad as metathoracic which supposedly belongs to the late instar larva. The wing pad is about 2 mm longer then the estimated length of the fore wing pad (specimen 3115/153) and more than 2x longer than the younger hind wing pad (specimen 3115/167).

When compared with the previous hind wing pad (specimen 3115/167), the RP of both wing pads ends with three branches on the posterior wing pad margin. However, the CuA of the smaller specimen 3115/167 is distally branched, while in the present wing pad it is simple, while the CuP is distally branched. MA is simple as in all other wing pads of the previous instars.

Considerable variability in the branching of MP, CuA and CuP and also in the position of the divergence points of RA/RP and MA/MP can be observed between the described hind wing pads.

Presumed subimaginal wings of *T. guttata*

Specimen no. 3115/151

Sinitshenkova 1979, p. 197, fig. 3a

Description. Wing completely preserved of oval shape; costal area broad proximally; numerous cross-veins present in the costal area and in the area between R and A of the wing; ScP parallel to R; RP diverging from RA at about 1/3 of the wing length; RA simple, curved towards the apex, ending on it; RP straight, distally pectinated, ending with four branches on the posterior margin; first RP branch diverging at 2/3 of the wing length (36 mm from wing base), at about half the length of RP; M stem running close to R stem, then diverged slightly, divided into MA and MP just before the point of divergence of RA/RP; MA and MP simple, ending on the posterior wing margin; Cu stem runs distant from stem M, diverges into CuA and CuP at about 8.5 mm from the base; CuA and CuP both simple, ending on the posterior margin; anal area with six veins preserved, all simple, rather straight.

Dimensions. Wing length 56 mm, maximum width 27 mm.

Remarks. The wing could not be re-examined. According to the figure of Sinitshenkova (1979), it is a well-preserved wing of oval shape and the costal area is rather broad, therefore it is

probably a fore wing.

The wing is about 4 mm longer than the hind wing pad specimen 3115/53. Sinitshenkova (1979) supposed that the fore wing belongs to a subimaginal instar of *T. guttata.*

On the figure of Sinitshenkova ScP seems to join RA closer to the mid-wing, than in other wing pads studied. In contrast to the fore wing pad of presumed later instar larva (specimen 3115/153) the MP is simple and the point of divergence of RA/RP is more distant from base than the point of divergence of MA/MP. The vein RP is ending with four branches, thus, with one more branch than in the fore wing pad of an early instar larva (specimen 3115/29).

Specimen no. 3115/63 (Fig. S1G)

Sinitshenkova 1979, p. 197, fig. 3b; plate VII, fig. 6

Description. Fragmentary wing; wing membrane dark with light spots; anterior and posterior margins slightly curved; costal area broad, with numerous cross-veins; wing venation well developed; main longitudinal veins discernible, corrugated; numerous cross-veins present in the area between the ScP and CuP; ScP concave, straight, running parallel to R, reaching RA at about 2/3 of wing length (approximately 47 mm from wing base); stem R forked into RA and RP at almost 1/3 of wing length (19.5 mm form wing base); RA convex, simple, distally curved, running close to anterior margin, ending on the wing apex; RP concave, pectinated, ending in four branches on the posterior wing margin; first branch of RP diverges at mid of wing length; M stem running close to R, MP diverging from MA just before the point of divergence of RA and RP; MA convex, simple, slightly curved, connected with R4 near wing margin; MP concave, simple, ending on the posterior wing margin; Cu stem not preserved; CuA convex, CuP concave, both vein precursors straight, simple, without preserved their terminal parts; anal area rather broad with six simple branches.

Dimensions. The wing length 66 mm, maximum wing width 32.7 mm.

Remarks. The wing is partially damaged, the base is missing, and the posterior margin is poorly preserved with medial part missing. The wing is darkly coloured with light lines along the well-developed veins and cross-veins, light spots in the centre of membrane cells and darkened costal area. The wing has a broad costal area; thus, it is probably a fore wing.

Sinitshenkova (1979) described the fore wing as subimaginal instar of *T. guttata*. It is about 10 mm longer than the previous fore wing (specimen 3115/151). The main difference from the larval fore wing pads and the second subimaginal fore wing (specimen 3115/151) is the topology of branching of RP, with forked first and second branch. The positions of the points

of divergence of RA/RP and MA/MP are the same as in the subimaginal wing specimen 3115/151. This wing differs from all the other wings and wing pads by the MA being distally attached with the psoterior branch of RP.

Specimen no. 3115/152 (Fig. S1H)

Sinitshenkova 1979, p. 197, fig. 3c; plate VII, fig. 5

Description. Incomplete wing; anterior margin straight, posterior curved; wing membrane dark with light spots; costal area narrow with few cross-veins; main longitudinal veins present; numerous cross-veins present in the area between RP and A; ScP concave, straight, running parallel to R; RA/RP diverged at 1/3 of wing length; RA convex; RP concave; M stem runs parallel to R, MP diverging from MA just before the point of divergence of RA and RP; MA convex, straight; MP concave, straight, distally bifurcated, ending on the posterior wing margin; Cu stem runs remotely from M, curved, CuA and CuP divergence at 10 mm from presumed wing base; CuA convex, CuP concave, both vein precursors simple, straight; anal area broad with eight branches, third one is distally bifurcated.

Dimensions. Length of incomplete wing 40 mm, estimated wing length about 57 mm, maximum wing width 27 mm.

Remarks. The fossil is fragmentary with wing base missing, part of anal area and distal part of the wing. The wing is darkly coloured, with light lines along the wing veins and light spots in the middle of the membrane cells. The costal area is relatively narrow, anterior margin straight and anal area broad, so it is probably hind wing.

Sinitshenkova (1979) considered the fossil as hind wing of subimaginal instar of *T. guttata*. The estimated wing length is about 5 mm longer than the length of the biggest larval hind wing pad (specimen 3115/53). The wing differs from the larval hind wing pad by having simple CuP, the distal part of the RP is not preserved.

The position of the points of divergence of RA/RP and MA/MP in all three subimaginal wings is the same with the RA and RP divergence point being more distant from the wing base. In contrast to the subimaginal fore wing (specimen 3115/151) this hind wing has a distally forked MP.

Imaginal wings of *T. guttata*

Specimen no. 3115/66

Sinitshenkova 1979, p. 194, fig. 1a; plate VII, fig. 7

Description. Incomplete wing of adult; anterior margin curved, posterior margin straight; costal area broad, with many cross-veins; dense network of cross-veins in the area between R and A; ScP running parallel to R, reaching RA at about 60 mm form the presumed wing base (6/7 of wing length); basal part of Radius not preserved; RA simple, slightly curved distally; RP pectinated distally, with three preserved branches; distal parts of RA and RP, not preserved; first RP branch diverged at about 2/3 of the presumed wing length; stem M runs posteriorly from R, MP diverging from MA proximally, but after the point of RA/RP divergence; MA curved towards the posterior margin, simple; MP forked distally, each branch ending with short fork on the posterior margin; Cu stem and proximal parts of CuA and CuP not preserved; CuA and CuP curved and ending on the posterior wing margin, CuA ending with short fork; anal area not preserved, only distal part of 1A figured.

Dimensions. Incomplete wing length 50 mm, maximum width 30 mm, estimated total wing length about 70 mm.

Remarks. The fragment of wing is missing proximal part of the wing and the wing tip. The costal area is rather broad; therefore, it was probably a fore wing.

Sinitshenkova described the wing as imaginal fore wing of *T. guttata* and estimated its full length on 80 mm. Based on the distances of the various points (distance of the first RP branching from the wing tip, distance of the MA/MP divergence point from the base and wing tip etc.) on the subimaginal fore wings, the full wing length was estimated about 70 mm. Thus, the wing would be about 4 mm longer than the longest supposedly subimaginal fore wing (specimen 3115/63).

The main difference from the bigger subimaginal fore wing (specimen 3115/63) is the pectinated RP, the forked MP and from the smaller subimaginal fore wing (specimen 3115/151) it differs in the forked MP. The cross-vein network is denser than in the subimaginal wings.

Specimen no. 3115/191

Description. Fragment of imaginal wing, only the base and proximal parts of wing preserved; posterior margin curved; costal area broad, with some cross-veins; dense cross-veins network in M-A area; ScP running parallel with R; RA diverged from RP approximately at 1/4 of the

estimated wing length (18 mm from wing base); M stem running close to R, MP diverging from MA at about the same distance as RA/RP divergence point; Cu stem more distanced from M, divided into CuA and CuP proximally at about 9 mm from the wing base; anal area broad, with five veins.

Dimensions. Length of incomplete wing about 26 mm, width about 25 mm; estimated full wing length about 70 mm, wing width about 30 mm.

Remarks. The fossil could not be re-examined; thus, it was described based on the figure of Sinitshenkova (1979). The fossil is fragmentary, only the base and part of the proximal wing area are preserved. The anterior margin is poorly preserved, but the costal area is rather broad, therefore, it is probably a fore wing.

Sinitshenkova described this fossil as a fragment of imaginal fore wing of *T. guttata*. The relative positions of divergences RA/RP and MA/MP are not preserved. The proximal parts of the ScP, R, M and Cu veins have the same course as in other wings and wing pads of the species.

Specimen no. 3115/68 (Fig. S2A)

Sinitshenkova 1979, p. 196, fig. 2a

Description. Distal part of imaginal wing; anterior margin straight, posterior margin strongly convex; costal area proximally broad, bearing numerous cross-veins; corrugation of the main veins preserved; many cross-veins between main veins present on the whole wing, creating network, many cells with lighter central spots and membrane peeled off; ScP concave, running close to R, joining RA about 15 mm from the wing apex (about 4/5 of the presumed wing length); RA convex, simple, curved towards the wing apex, ending on it; RP concave, pectinated, ending with four branches on the posterior wing margin; first RP branch separates at about 28 mm from wing apex (about 3/5 of assumed wing length); MA convex, simple, curved, ending on the posterior wing margin; MP concave, forked, each branch ending on the posterior wing margin with short fork; CuA convex, simple, ending on the posterior margin; CuP concave, ending on posterior margin with short branch; only small part of anal area preserved, with distal parts of two anal veins, 1A ending on the posterior margin with two branches.

Dimensions. Length of incomplete wing about 45 mm, fossil width 28.5 mm; estimated full wing length about 70 mm.

Remarks. The fossil is a distal part of imaginal wing. The membrane of the wing is wrinkled,

thin, without apparent colouration. Middle parts of many membrane cells with light circular spots or membrane peeled off. Since the anterior margin of the wing is rather straight and costal area narrow, it is presumably hind wing.

Sinitshenkova described the wing as a hind wing of imago of *T. guttata* and estimated its full length to be about 80 mm. Based on the ratio between the distance of the point of CuA ending from the wing base and the estimated wing length of the subimaginal wing specimen 3115/152, the full length of the imaginal hind wing was estimated about 70 mm.

In contrast with the presumed subimaginal hind wing (specimen 3115/27) the MP is forked. The pattern of venation corresponds to the pattern of fore wing in imago (specimen 3115/66) with pectinated RP, MP ending with two branches, each with short terminal fork and CuP ending with short terminal fork on the posterior margin.

Specimen no. 3115/193

Sinitshenkova 1979, p. 196, fig. 2b

Description. Incomplete imaginal wing with anterior part preserved; anterior margin proximally straight, distally curved towards the wing apex; costal area narrow with several cross-veins; numerous cross-veins forming network between main veins; ScP straight, simple, running next to R, joining RA near wing pad apex; RA diverged from RP at about 1/3 of the presumed wing pad length (about 50 mm form assumed wing apex); RA simple; RP pectinated, with three preserved branches; first RP branch diverging at about half of the length of RP; M stem running parallel to Radius, than diverged posteriorly, point of separation of MA and MP not preserved; MA ending with small bifurcation on the posterior wing margin.

Dimensions. Length of the incomplete wing 50 mm, width about 19 mm; estimated wing length about 70 mm.

Remarks. The fragmentary wing is missing the proximal part, most of the posterior margin and the wing tip. The main veins are only partially preserved, with their posterior parts missing, the CuP and anal area are not preserved. The costal area is narrow, thus it corresponds to hind wing. Sinitshenkova (1979) also considered the fossil to be fragment of the hind wing of adult. The length of the full imaginal hind wing is estimated based on the estimated length of hind wing specimen 3115/68. The preserved venation also corresponds to the pattern of the hind wing found in specimen 3115/68 with four branches of RP.

Genus *Paimbia* Sinitshenkova, 1979

Type species. *Paimbia fenestrata* Zalessky, 1979

Paimbia fenestrata Sinitshenkova, 1979

Sinitshenkova (1979: p. 198, fig. 8, 9, 10; plate VIII, fig. 1, 2, 3, 4, 5): Palaeodictyoptera: Tchirkovaeidae

Holotype: PIN 2293/4, subimago.

Holotype location: Paymbu River locality, Tunguska Basin, Krasnoyarsk Territory, Russia; Late Carboniferous, Kasimovian, Kata Formation

Re-examined material:

PIN RAS Moscow collection: 2293/4 (holotype), 2293/6, 2293/7, 2992/1

Specimen no. 2293/6; part and counterpart (Fig. S2B,C)

Sinitshenkova 1979, p. 204, fig. 10 a, b, c

Description. Exuvia of a relatively large larva, preserved mouthparts modified into rostrum, part of metathorax with attached partly preserved left fore wing pad, metathorax with left hind wing pad with broad base, both wing pads directed obliquely backwards, thorax and first two abdominal segments with a median longitudinal line clearly discernible, abdomen with ten abdominal segments, with discernible ovipositor on segments VIII and IX and cerci on the last abdominal segment.

Mouthparts: stout mandibular stylets, shorter and thinner maxillary stylets and long multisegmented maxillary palpi are recognizible.

Left posterior part of mesothorax with left fore wing pad attached; the wing pad poorly preserved; metathorax rectangular, with two posterior transverse sulci, bears broadly attached hind wing pads, left wing pad partially obscured by fore wing pad, base and anal margin of right hind wing pad preserved.

Left metathoracic wing pad partially preserved pattern of venational precursors, corrugation of the main precursors visible; distal part of the costal area narrow; RP concave, pectinate with

four branches, first branching of RP at about mid length of RP, MA convex simple, MP concave simple, CuA convex simple, CuP concave simple, three faint anal veins precursors discernible; anal area rather broad.

Base of the right metathoracic wing pad with preserved anterior and posterior fusion to the notum, medial articulation area with distinctly preserved anal brace and large axillar plate. Abdomen wide, twisted to the side, segments I-VI subdivided by transverse sulci, clearly discernible ovipositor exceeding the tip of the abdomen, a pair of stout annulated cerci on the last abdominal segment.

Dimensions. Body length about 50 mm; metathorax length 8 mm; metathoracic wing pad length about 17 mm, width 7 mm; abdomen length 35 mm, width 10 mm

Remarks. The pattern of developing venation in the metathoracic wing pad precisely corresponds to the adult venation of the genus *Paimbia* Sinitshenkova, 1979. The basal structures preserved on the right metathoracic wing pad correspond to that of isolated larval wing pad specimen 2293/7 (described below).

The wing pads are relatively small compared to the body-size, but the larva has preserved markedly long and well ovipositor, contradicting the assumption of Sinitshenkova (1979), that the specimen represents a middle instar larva of *Paimbia fenestrata*. Another significant character is the presence of piercing-sucking mouthparts.

Specimen no. 2293/7; part and counterpart (Fig. 22; Fig. S2E)

Sinitshenkova 1979, p. 204, fig. 10d

Description. Well preserved wing pad with enlarged outer margin, broadly attached to part of thoracic notum, the wing pad pointed obliquely backward in respect to the body; the notum posteriorly with two grooves, dividing it into three parts; discernible base with posterior and anterior fusion of the wing with notum, discernible anal brace and one large axillary plate; dense/thick cuticle?; wing pad elongated, narrow, anterior margin strongly convex, posterior margin concave; discernible corrugation of the main vein precursors; all vein precursors curved towards the posterior margin of the wing pad; costal area narrow; faint ScP concave, joins RA shortly after mid wing pad length; R arising from anteriorly of the axillary plate, RP diverges from RA in the middle of the wing pad length; RA convex, simple, ending on the wing pad apex; RP concave, first branch diverging in the mid length, RP ends with five branches on the posterior wing margin; first branching of RP at about mid length of RP; M stem arising further distanced posteriorly from R, running anteriorly for a while and curving posteriorly just before

meeting R; MA and MP diverging at about 2/5 of the wing pad length; MA convex, simple; MP concave, simple; Cu arising at about middle of the axillary sclerite, diverging into CuA and CuP at about 1/4 of the wing pad length; CuA convex, simple; CuP concave, simple; anal area broad, with six anal vein precursors arising from the anal brace.

Dimensions. Full fossil length 18 mm, wing pad length 14 mm, wing pad width 5 mm, length of notal fragment 6 mm, width 2.7 mm.

Remarks. The cuticle of the thorax and wing base seems very thick, covered with tubercles. The free margin of the thoracic notum corresponds to the median longitudinal line of the larval exuvia (specimen 2293/6), therefore the fossil probably represents part of larval exuvia. The wing pad would then, in fact, be the cuticular sheath, in which the wing developed before eclosion. This suggests that the inner structure of the developing hind wing in the wing pad was projected to the cuticular case, covering the developing wing.

Sinitshenkova (1979) regarded this specimen as an early instar larva. The wing pad is slightly smaller and the notal half preserved is narrower than in the exuvia (specimen 2293/6), therefore the fossil could represent a younger larval instar, with the same position in respect to the insect's body.

When compared to the more preserved metathoracic wing pad of the specimen 2293/6, the RP branching topology is different (similar to the topology of RP on the presumed subimaginal wing tip of *P. fenestrata*, specimen 2992/5 illustrated in Sinitshenkova 1979, p. 203, fig. 9c), but other than that the pattern of vein precursors is congruent and the wing base of the right metathoracic wing pad of the exuvia has the same structure as can be found in the present specimen.
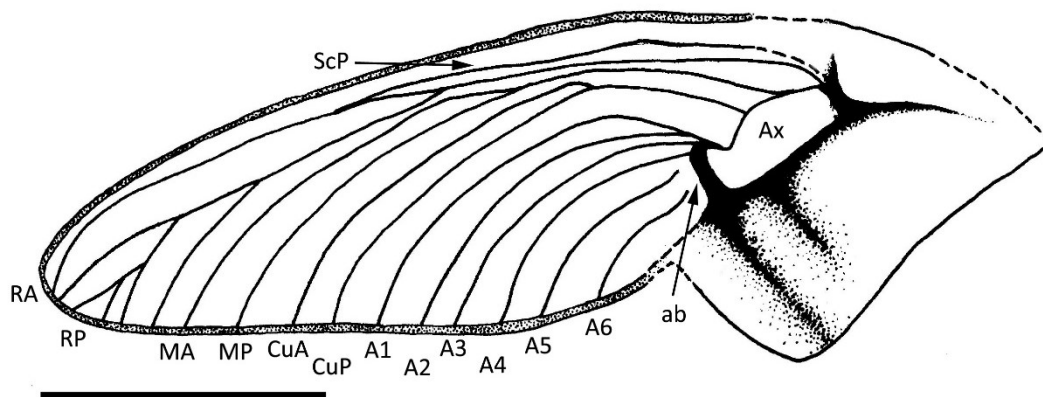
Figure 22: *Paimbia fenestrata*, line drawing of the specimen 2293/7. A1-A6, anal veins; ab, anal brace; Ax, axillary plate; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; RA, radius anterior; RP, radius posterior; ScP, subcosta posterior. Scale bar = 5 mm.

Specimen no. 2293/4 (holotype) (Fig. S2F)

Sinitshenkova 1979, p. 203, fig. 9 a, b

Description. Remnants of an winged stage of insect, head and thoracic segments distorted, prothorax with paranotal lobes, mesothorax bearing left fore wing with darkly coloured wing tip, the colouration begins with the point of the last branch of RP reaching the posterior margin, base of the right fore wing present, metathorax with both hind wings missing apical parts, all wings bent posteriorly, present parts of mesothoracic legs and one metathoracic leg, preserved five abdominal segments, nygma-like structures present in some membrane cells of the fore wing.

Mesothoracic wing: anterior margin convex; costal area narrow, with few cross-veins, darkly coloured; main longitudinal veins corrugated, numerous cross-veins in the area between R-A; ScP concave, reaching RA at mid-wing length; RA convex, ending on the anterior wing margin just before apex; RP diverging from RA at 1/3 of the wing length, concave, pectinated, ending with four branches on the posterior wing margin; first branching of RP at about mid length of RP; M stem arising more remote form R, running anteriorly towards R, then diverging backwards just before meeting it; MA and MP diverging at 1/4 of the wing pad length; MA convex, simple; MP concave, simple; Cu arising close to the M stem, running anteriorly for a while, then curving backwards; CuA and CuP diverging just slightly closer to wing base than MA and MP divergence point; CuA convex, simple; CuP concave; anal area with three preserved anal veins.

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Metathoracic wings: missing tips, the wing basal parts are distorted (twisted or compressed together); costal area narrow, darkly coloured; main veins corrugated; RA convex, simple; RP concave, pectinated with four branches; MA and MP diverging at about 1/4 of the wing pad length; MA convex, simple; MP concave, simple; CuA partially preserved, convex.

Mesothoracic leg: preserved distal part of tibia, tarsus with four segments, ending with claw.

Metathoracic leg: preserved femur and tibia, proximal part of tarsus.

Abdomen: preserved five segments, sixth only partly preserved, nota divided by transverse sulci.

Dimensions. Length of preserved parts of body 52 mm; length of fore wing 46 mm, width of fore wing at base 9.6 mm; estimated length of hind wing 46 mm; abdomen width about 10 mm.

Remarks. Sinitshenkova (1979) described this specimen as subimago of *P. fenestrata* based on the wings being curved posteriorly and slightly shorter (4 mm) than adult wings of the species. The pattern of main longitudinal veins corresponds to the pattern of the main longitudinal vein precursors in the assumed middle larval instar larva (specimen 2293/6), but the topology of the RP branching differs from that of the isolated smaller wing pad (specimen 2293/7).

Specimen no. 2992/1 (Fig. S2D)

Sinitshenkova 1979, p. 202, fig. 8a

Description. Fossil part of adult wing preserved, missing wing tip, posterior margin and part of wing base; part of axillary plate preserved; anterior margin slightly convex; costal area narrow, without cross-veins, darkly coloured; the area between R and 4A with numerous cross-veins, variably oriented, some undulated; nygma-like structures present in some membrane cells; ScP concave, runs parallel and close to R, ends on the costal margin near the point of the first divergence of RP; R arising just anteriorly of the axillary plate, running straight; point of divergence of RA and RP about 16 mm form the wing base, about 1/3 of the assumed wing length; first branching of RP preserved; M stem arising closer to the middle of the axillary sclerite, running anteriorly to R, then diverging posteriorly, point of divergence of MA and MP at about 1/4 of the estimated wing length; MA simple, convex; MP simple, concave; Cu arising closer to M stem, runs anteriorly for a while, then diverging posteriorly; CuA and CuP diverging at about 1/5 of the estimated wing length; CuA, convex, simple; CuP, concave simple; anal area broad with four anal veins preserved.

Dimensions. Length of preserved wing 42 mm, estimated full wing length about 50 mm.

Remarks. The darkly coloured distal part of the wing fragment can be result of preservation, and if not, it certainly does not correspond to the colouration of the wing tip found in the specimen 2293/4 nor of the specimen of imaginal hind wing of *P. fenestrata* shown in Sinitshenkova (1979, plate VIII fig. 1, specimen 2293/1), since there the colouration of the wing tip begins with the point where the last branch of RP meets the posterior wing margin, whereas the colouration in this specimen begins shortly after the first bifurcation of RP.

Sinitshenkova (1979) regarded it as fore wing, since the anterior margin of the fore wing of *Paimbia fenestrata* should be straight, while the anterior margin of hind wings is concave. But the specimen represents only the proximal part of the wing, and a considerable part of the distal and posterior wing areas is missing. In fact, the specimen could also represent the more proximal part of hind wing when compared with the distal part of imaginal hind wing preserved in the specimen 2293/1.

The general pattern of venation corresponds with that of the fore wing of specimen 2293/4, but there are some differences. The MA is bent more subtly in the present specimen and remote from the point of divergence of MA and MP, while in the fore wing of the specimen 2293/4 the MA is much more sharply curved close to the proximity of the divergence of MA and MP. The cross-veins in some areas have different orientation. In the present specimen, the cross-veins between MP and CuA, CuA and CuP are curved and positioned obliquely to the longitudinal veins, while in the specimen 2293/4 they are straighter and are positioned rather transversally to the longitudinal veins.

3.2. The wing development in *Cloeon dipterum* (Ephemeroptera: Baetidae)

In the earliest larva examined (specimen j010), the development of the wings already begun. The evaginations of the thoracal integument were noticeable on the dorsal lateroposterior edges of the meso- and metathorax and had the appearance of small sack-like structures. A single weak trachea form the anterior stem of the leg trachea was approaching the evagination, but did not seem to enter it. The surface of the wing pad cuticular case was covered by small tubercles. The thoracic nota and the wing pads were darkly pigmented (Fig. S4A).

During the next stages of development (specimen j005) the mesothoracic wing pads were elongated and the anterior tracheal trunk was clearly visible in the mesothorax, where it approached the developing wing pad, while in the metathorax there was no such structure recognizible (Fig. S4B).

When length of the wing pads reached to about middle of the metathorax (specimen j012) one big basal lacuna became discernible at the base of the mesothoracic wing pad, with the anterior tracheal trunk entering it. From the basal lacuna few longitudinal lacunae permeated the through of the wing pad and separate tracheae projecting from the anterior tracheal trunk and seemed to enter some of them. Also the circumambient putative costal lacuna was discernible near the wing pad margin. The metathoracic evaginations were just slightly longer than in the earliest stage of development, otherwise they remained unchanged (Fig. S4C).

In the more advanced wing pads (specimen j017), reaching about 2/3 of the length of the metathorax, the costal, subcostal, radial anterior and radial posterior, medial and cubital lacunae were discernible. The transverse channel which would become the costal brace in the subimaginal and adult wing was clearly visible. The RP lacuna was divided into two branches. The C, ScP, RA and RP lacunae were connected by transversal channels, precursors of the future cross-veins. The pattern of lacunae already agreed to the general pattern of adult venation, only the cubitus posterior and anal area was not discernible. The tracheal trunk divided into branches, one seemed to enter the ScP lacuna, another entered the RP lacuna, and one trachea entered the median lacuna and one the cubital. The trachea entering the RP lacuna divided, the anterior branch ran through the first branch of RP lacuna, which will become the first RP branch in the adult wing, while the posterior branch ran through the last branch of RP lacuna, which later becomes the last branch of RP in the adult wing (Fig. S4D).

In the following stages (specimens j016, j019) (Figs S5A, B) the mesothoracic wing pads extended over the non-developing metathoracic rudiment and posterior margin of the metathorax. The general pattern of venation in adults was discernible in the pattern of lacunae. The M lacuna divided into MA and MP lacunae, the trachea that entered the M lacuna

previously now ran through the MP lacuna; the Cu lacuna divided in to CuA and CuP lacuna. The CuP lacuna was located on the border of the wing pad, where the wing sheath merged with tergum. The cubital posterior and anal area were for the first time noticed in the larva of approximately the same age (specimen j020) and it was located beyond the border of the wing pad under the mesothoracic notum, divided from the surrounding structures by the costal lacuna and a thickened line of cuticle (Fig. S5C).

In older larva (specimen j022) the fourth branch of trachea is clearly distinguishable for the first time entering the CuP lacuna. The transversal lacunae, that would later form the subimaginal and adult cross-veins are discernible in the area between costa and media posterior. Distally, between the first and last branch of RP lacuna and between the MP and CuA lacunae, were present the intercalary lacunae later becoming the longer intercalary veins of the subimaginal and adult wings (Fig. S5D).

The wing pads of the last larval instar reached the middle of the first abdominal segment and the lacunae agreed to the pattern of subimaginal wing venation (specimen j025). All main longitudinal lacunae and their branches and transversal channels, i.e. future cross-veins, were discernible. The only missing features were the shorter intercalary veins present on the posterior wing margin between the main longitudinal veins and the longer intercalary veins endings in the subimgainal and adult wings. The MP trachea sent a thinner trachea to the CuA1 lacuna through transversal channel, which would later become a cross-vein between MP1 and CuA1. The circumambient costal lacuna separated the wing pad from the thickened margin created by the line of hypodermis and thickened cuticle (Fig. S6A).

Looking at the outer surface of the wing pad of the last larval instar right before moulting (specimen j001), when the developing wing elongates and becomes crumpled inside the cuticular wing sheath, the corrugation of the main vein precursors is clearly distinguishable. The costal brace is already present (Fig. S6B).

Within each single instar stage, the lacunae are not discernible in the whole area of the wing pad at the same moment, as only in one specimen of approximately middle instar larva (specimen j020) the cubital anterior and posterior and the anal lacunae could be successfully distinguished, while in the specimens of older larvae the cubito-anal area was not found differentiated. For the whole time of development the wing pad is fully fused with the thoracic notum.

The subimaginal wings (specimen j033, Figs S6C, D) are about three times longer than the wing pads of the last larval instar. The wings have well developed wing veins, all longitudinal veins and the longer and shorter intercalary veins running from the posterior margin are already

present. The cross-veins are less pronounced than in the imaginal wing and in the costal area most of them is missing, even though their lacunae were clearly discernible in the larval instars. The wing is translucent and row of long setae is present on the posterior margin. The whole surface of the wing membrane including the veins is densely covered with short microtrichia. The imaginal wing is the same size as the subimaginal one. It differs from the subimaginal wing in transparent iridescent membrane and the darkened brownish area between the RA and costal margin. Also the cross-veins are better developed and the cross-veins in the costal area are well discernible. The wing membrane is thin and without setae.

4. Discussion

Tchirkovaea guttata was first time described by Zalessky (1931) based on a fragment of fossil wing from North Oungua river, Tcheremitchkino, Kousnetzk basin, Russia. The species was placed in the family Diaphanopteridae, which was at that time considered to belong into the order Megasecoptera. Zalessky regarded the wing as imaginal with estimated length of 56 mm. The wing was rather oval, the ScP was reaching the RA distally, there were nine cross-veins in the costal area, the RA was curved to approach the wing apex, the RP diverged at about one third of the wing length and was pectinated with three terminal branches. Between the main veins were cross-veins arranged in various angles. Rounded light spots were present in numerous cells. Zalessky did not mention at all, why he placed *T. guttata* among Diaphanoptera. From the figure in Zalessky (1931, p. 215 fig. 4) it is clear, that the MA, MP, CuA and CuP were all simple and that the point of divergence of MA and MP was more distant from the wing base, then the point of diversion of RA and RP. Sinitshenkova (1979) regarded the holotype described by Zalessky (1931) a subimaginal wing because of its size, shape and the simple cross-veins, however the author could only rely on the figure and description in the original paper.

Sinitshenkova (1979) later established a separate family Tchirkovaeidae and re-assigned it to the Palaeodictyoptera based on the differences between fore and hind wings, higher number of anal veins, broad wing base, branching cross-veins or archedictyon and the absence of connections of the basal parts of the veins. The family Tchirkovaeidae can be distinguished from other families of the order by shorter and broader wings, fewer longitudinal veins, and simple veins MA, CuA and CuP. But the last character does not apply to some of the supposed wing pads of *T. guttata*, as in the hind wing pad of the supposed early larva (specimen 3115/160) the CuA and CuP are both forked, in the hind wing pad of the middle instar larva (specimen 3115/167) CuA is branched, and in the hind wing pad of later instar larva (specimen 3115/53) the CuP is branched. Only the longitudinal vein MA is simple in all re-examined wings and wing pads.

One of the indirect evidence to place Tchirkovaeidae within Palaeodictyoptera is, according to Sinitshenkova (1979), the sole discovery of the series of larval, subimaginal and imaginal remains, since the larvae of Diaphanopterodea are not known and their ecology could greatly differed from the adult one, so they are not found together.

Following the reassignment of *Tchirkovaea sharovi* to the family Breyeriidae as *Breyeria sharovi*, Sinitshenkova (1981) added new characters to the diagnosis of the family Tchirkovaeidae: MP simple or with a short terminal fork, M and Cu branches curved only

weakly distally, hairs sometimes present on the wing membrane or along margin, but hair tufts always absent from membrane.

The family Tchirkovaeidae comprises two genera, monotypic *Tchirkovaea* Zalessky, 1931 is distinguished form *Paimbia* Sinitshenkova, 1979 by the more pronounced heteronomy of the fore and hind wings, more oval wing shape, broader costal area, and branched MP in the adult wing (Sinitshenkova 1979). *Tchirkovaea guttata* should be distinguishable also by the colouration pattern of light spots on the wing membrane, which can be observed on the late larval instar wing pads, subimaginal and imaginal wings (less pronounced). The archedictyon (ancient type of dense network of cross-veins) on the wings of *T. guttata* is formed by large cells, while in the wings of *Paimbia* it is absent (Sinitshenkova 1979).

Sharov (1973) previously assigned the fossil specimens of *P. fenestrata* (2293/6, 2293/7 and 2293/4) to the genus *Brodioptera* Copeland, 1957 (family Brodiopteridae) and considered this family to belong in Palaeodictyoptera. It is not clear, which characters present could have led Sharov (1973) to consider the placement within the family Brodiopteridae. But the placement of the specimen 2293/4 to the genus *Brodioptera* could not be accurate, since the Brodiopteridae show numerous characteristics typical for the members of order Megasecoptera, such as the lack of the prothoracic lobes, which are present in the specimen 2293/4, and the wings of Brodiopteridae have tendency to form a narrow stalk proximally (Carpenter 1992, Sinitshenkova 1979).

Sinitshenkova (1979) mentioned an almost complete insect *Psychroptilus burrettae* Riek, 1976, which shared many features with the genus *Paimbia*. Riek (1976) decided to designate a separate family Psychroptilidae and assigned it into the order Megasecoptera, because the specimen lacked the prothoracic lobes. Since then the family was transferred to Palaeodictyoptera (Carpenter 1992). The wing venation of *P. burrettae* is very similar to that of *Paimbia*. The ScP ends on the costal margin, which can be also found in the adult wing of *P. fenestrata* (specimen 2992/1).

The pattern of developing venation of wing pads and adult venation of wings in *Tchirkovaea guttata* is highly variable in the terms of the number of branches of the main veins or vein precursors and their branching topology. The RP is pectinated in all specimens where it is preserved, with three to five branches, and note should be mentioned to the different branching topology in the specimen 3115/63 with the subimaginal fore wing. The MP is simple in some specimens, while in others it has from two to three branches. CuA and CuP are usually simple, but in few specimens are found branched.

The only main veins that appear in the same state in all specimens of *T. guttata* are the ScP and

RA, and MA. The ScP always enters the RA distally, the RA is always simple and ends on the wing apex and MA is always simple, ending on the posterior margin. In one specimen (3115/155) the M stem is not preserved or it was not even present and the MA runs along RA and RP for quite a long distance, while in other specimen No. 3115/63 the MA merges with the last branch of RP distally. The pattern of arrangement of the basal portions of the main veins stays constant in all wing pads and wings, with M running close to R and then curving posteriorly and Cu arising somewhat more distant from M stem, running obliquely forward for a little while and then curving posteriorly. Another feature common for all wings and wing pads is the divergence of the RP from RA at about one third of the wing or wing pad length.

With regard to the above mentioned differences in the pattern of developing wing venation and venation of supposed subimaginal and adult wings, it is questionable whether all specimens assigned to the species *T. guttata* by Sinitshenkova (1979) can be placed into one species. Sinitshenkova (1979) clissify the specimens in order according to the size of the wing or wing pad from the smallest to the largest and assigned them to five different categories, early, middle, late instar larva, subimago and imago. One would assume, that the number of branches of each vein would grow gradually through the development or stay unchanged at least, this was also observed in the ontogeny of *C. dipterum*, where the main lacunae appeared and differentiated progressively throughout the wing development. But after a closer look at the variation of the pattern of developing venation in the course of supposed development of the *T. guttata* it is evident, that this is not the case here (Tab. S3).

Sinitshenkova (1979) followed earlier authors (Sharov 1973; Kukalová 1969; Kukalová-Peck & Peck 1976) and assumed, that this series of wings and wing pads of *T. guttata*, together with the larva and supposed subimago of *P. fenestrata* supports their view on the postembryonic development in the order Palaeodictyoptera. The author proposed the following scenario of the development, called archemetaboly: the larval stages had developed venation not differing much from the adult one, then followed two subimaginal instars with well-developed wings capable of flight and finally imago with completely developed wings. Through the course of the postembryonic development the wing pads were supposed to gradually enlarge, their bases to become narrower and the axillary sclerites to appear, so that the wing pads could become movable. The orientation of the wing pads was also assumed to change in the course of the development from obliquely backward to perpendicular position to thorax.

Contrary to this hypothesis, evidence was presented in this thesis, that the smaller specimens in this fossil series do most probably represent the developing wing pads similar to that of recent insects. The enlarged outer margins correspond to the margins of the wing pads of *C. dipterum*,

created by the thickened cuticle and epidermis divided from the rest of the wing area by the costal lacuna. This together with the creased surface of the fossil wing pads clearly indicates presence of the cuticular case or wing sheath covering the developing wing inside. The inner structure was probably similar to the structure of the wing pads we know in recent hemimetabolous insects with systems of lacunae forming between two layers of the epidermis and tracheae and nerves running through them (Prokop et al. 2016, 2017a,b).

When compared to the state of development in the earliest instars of *C. dipterum*, with its barely noticeable evaginations of the integument lacking lacunae and tracheae, the pattern of developing venation and corrugation in the smallest specimen of *T. guttata* (specimen 2854/2) wing pad already agreeing with the general venational pattern of *Tchirkovaea* with the RP branched. This would suggest, that it could represent rather a middle or later instar larva. The second smallest specimen of *T. guttata* (specimen 3315/29) with the pattern of developing venation and corrugation discernible could then be regarded a late larval wing pad, and given the already well developed cross-vein precursors, it could represent one of the last larval instars or even the last one. For comparison, in the last larval instar of *C. dipterum* the cross-vein channels of the wing pad are present and on the surface of the wing pad the corrugation of the main longitudinal vein precursors is clearly discernible

Based on the hypothesis of gradual growth and change of orientation of the wings Sinitshenkova (1979) assumed, that the larval exuvia of *P. fenestrata* (specimen 2293/6) was that of a middle larval instar, since it had wing pads relatively small compared to the body and they were oriented obliquely backwards. But the presence of well-developed ovipositor highly contradicts the assumption and suggests that the specimen represents a late instar larva and maybe even the exuvia of the last larval instar (see also Prokop et al. 2015). Also, in the specimen 2293/7 the position of the wing pad towards the thorax is the same as in the specimen 2293/6, but the former is smaller, which, if we assume, that these are the same species, contradicts the idea of the gradual change of orientation in the course of ontogeny. However, the wing pad of the younger larva (specimen 2293/7) differs from the wing pad of the putatively later larval instar (specimen 2293/6) and also the wing of the assumed subimago of *P. fenestrata* (specimen 2293/4) in the topology of the branching of RP, and it differs from the imaginal wing of *P. fenestrata* (specimen 2992/1) in the ScP ending on RA, not on costal margin, so it is not sure, whether these specimens even belong to the same species.

It can be assumed, that the course of development of the wing pads was similar in the two probably closely related genera *Tchirkovaea* and *Paimbia*. Given that the imaginal wings of *Paimbia* are estimated to be around three times longer than those of the putative last larval wing

pads (specimen 2293/6), a similar relationship should be found between the wing pads and wings within the species *Tchirkovaea* and this relationship does seem to apply in *Tchirkovaea* for some of the wing pads and their probable corresponding imaginal counterparts. When this information is compared with the different patterns of venation of the putative wing pads and subimaginal and imaginal wings of *T. guttata*, it is possible to differentiate multiple potential species (Tab. S3). Also in the *C. dipterum* the ratio of the length of the adult wing and the last larval wing pad was found to be about 3:1.

Some of the evidence given by Sinitshenkova (1979) for the presence of the subimaginal instars in the ontogeny of Palaeodictyoptera was the darkened membrane of the supposed subimaginal wings of *T. guttata*. She compares it to the dense membrane of the immature wing pads and states, that the colouration is caused by the retention of the epidermal layers in the subimaginal wing essential for the moulting. It is true, that the subimaginal wings of *C. dipterum* are not transparent (contrary to the imaginal ones), but translucent, which is most probably caused by the present epidermal layers (but also the dense coverage by microtrichia), but there is no other evidence than the colouration, that could prove that this is the case also in the supposed subimaginal wings of *T. guttata*.

Another distinctive character of the subimaginal wings should be the underdeveloped cross-veins, which were always simple and weak compared to the longitudinal veins in the subimaginal wings, whereas in the imaginal wings the cross-veins were well developed and could be branched, creating the archedictyon. The cross-veins in the subimaginal wing of *C. dipterum* are weaker than in the imaginal wing, but all of the future imaginal cross-veins are already present in the wing (apart from some cross-veins of the costal area). Moreover, at least in the supposed subimaginal wing specimen 3115/63 the cross-veins are strong and well developed.

After comparison of the three supposed subimaginal wing specimens two distinct venational patterns can be observed. The specimen 3115/63 was about 10 mm longer than the other subimaginal fore wing (specimen 3115/151) and shows two major dissimilarities: (1) the completely different topology of the branching of RP than cannot be seen in any other specimen of *T. guttata* studied in this thesis and (2) the MA and the last branch of RP being connected near the wing margin. The difference in sizes of the two specimens of supposed subimaginal wings was probably the reason, why Sinitshenkova (1979) assumed, that there were at least two subimaginal instars in the course of development of *T. guttata*, however, based on the distinct venational pattern the specimen 3115/63 could be well regarded to represent imaginal wing of representative of a separate species.

Furthermore, the pattern of venation of all three supposed subimaginal wings differs from the imaginal one in one important character, which is the position of the points of divergence of RA/RP and MA/MP. While in the subimaginal wings the point of divergence of MA and MP lies closer to base of the wing, in the imaginal wings it is the other way round. Also the size of the wings would need to change in the final moult, since the supposed subimaginal wings are about 4 to 14 mm shorter than the imaginal wings, according to the estimated wing length of the imaginal wings being 70 mm in this thesis, while Sinitshenkova estimated the length to be 80 mm, so that the change in size would have needed to be even greater. For comparison, in the recent mayfly *C. dipterum* the subimaginal wings have already developed all the cross-veins (except for some in the costal area) and branches of longitudinal veins present later in the imago. The presence of the subimaginal instar in *P. fenestrata* is challenged by the differences in the venation (different appearance of MA, different orientation of cross-veins between MP and CuA, CuA and CuP) and colouration between the wing of the putative subimaginal instar (specimen 2293/4) and the imaginal wing (specimen 2992/1).

Sinitshenkova (1979) suggests, that the archemetaboly in its full form did not persist to present, because the underdevelopment of wings of the juvenile forms did not allow them to escape from predators. The author proposed that to avoid escape this pressure the mayfly larvae sought the protection of aquatic environment and the subimago of recent mayflies is remnant of the supposed archemetaboly. It has been suggested, however, that the subimaginal instar of recent mayflies may be the adaptation for the emergence of the late stage from the water surface (or even underwater emergence of subimagos in some species of mayflies), as it was proved that the subimagos escape from water more easily than their adults and the microtrichia covering the surface of the subimaginal wing could provide this advantage (Edmunds & McCafferty 1988).

One of the distinctive features of *Tchirkovaea guttata* should be the absence of the so called "faceted organs", which could be the nygmata of recent holometabolous insects, but similar structures were observed in the early instar larva of *T. guttata* (specimen 3115/29). Structures that look very similar in appearance to the nygmata those can be found in some representatives of Mischopteridae (Megasecoptera), Cacurgidae (Archaeorthoptera), Diaphanopterodea and also in other representatives of Palaeodictyoptera (e.g., Carpenter 1951; Handlirsch 1911; Forbes 1943; Carpenter 1963; Engel et al. 2013; Prokop & Kukalová-Peck 2017). The structures preserved in the fossils of the Palaeozoic insects seem to be larger than those of recent holometabolous insects (Carpenter 1963), however, nygmata can also significantly differ in size among different specimens of the same species (Martynov 1924; Jolivet 1955; Londt 1974).

There are generally about 2-6 nygmata (6 on the fore wing of fossil representative of the hymenopteran family Xyelidae from Triassic; Wang et al. 2014) present on the fore or hind wing of representatives of some orders of Holometabola (i.e. Trichoptera, Mecoptera, some Neuroptera and Megaloptera, Hymenoptera: Symphyta) (Forbes 1924; Martynov 1924; Londt 1974; Minet et al. 2010). No nygma or nygma-like structure has ever been found in any recent hemimetabolous insects (e.g. Martynov 1924; Londt 1974; Wang et al. 2014).

These structures were also observed in another representative of the family Tchirkovaeidae, *Paimbia ultima* Sinitshenkova, 1981. The ESEM photographs of this specimen of *P. ultima* revealed, that the external appearance of the formations corresponds to the recent nygmata found in Holometabola (Fig. S3).

The function of nygmata in the recent holometabolans is not known. Martynov (1924) found out, that in Trichoptera, where the wings of imagoes retain the inner epidermal layers, each "facet" (circular formation of cellular origin, located on the elevated structure of the wing membrane, surrounded by radial grooves) corresponds to a single individual cell of the given epidermal layer. Since the nuclei of these elongated cells are much longer, than the nuclei of other epidermal cells, Martynov (1924) assumed, that these facetes were of secretory nature (see also Jolivet 1955, Kristensen 1989). In other groups of insects with nygmata, where the epidermal layers of the wing are not maintained to the imaginal stage, the structures are only cuticular remnants of the true nygmata and they lost their function (Martynov 1924).

Based on the similar appearance of the nygmata and the wax glands of Aphidoidea, Martynov (1924) suggested, that nygmata could perform a similar function in the past, i.e. the secretion of was or some wax-like substance (see also Jolivet 1955). Another frequently suggested function of the nygmata is sensory (Londt 1974; Nichols & Schuh 1989; New 1991). However, no innervation has ever been found in nygmata (Moretti & Dottorini 1965). Forbes (1924) suggested the possibility, that the nygmata can play role in the imaginal ecdysis by secreting a moulting fluid, but added, that there is no evidence for this assumption.

Minet et al. (2010) consider the presence of nygmata in the area between the radial and medial veins as an autapomorphy of all holometabolans. Nygmata in the specimen 3115/29 of *T. guttata* are present in this area. There are also other fossils of Palaeodictyoptera and also Megasecoptera with nygmata present in the cells between veins R and M, e.g. *Diaphanoptera munieri* Brongniart, 1893 (Prokop & Kukalová-Peck 2017), *Mischoptera nigra* Brongniart 1894 (Carpenter 1951) or *Lithomantis bohemica* (Novak, 1880).

The single large axillary plate found in the early larval fore wing of *T. guttata* (specimen 2854/2) was also found in the larval wing pad of *Paimbia fenestrata* (specimen 2293/7) and of

Parathesoneura carpenteri Sharov & Sinitshenkova, 1977 (specimen 3115/26), both were re-examined for comparison. Kukalová-Peck (1983) interpreted the basal structures of both specimens (*P. fenestrata*, 2293/7 and *P. carpenteri*, 3115/26) as arising from the presumed four rows of ancestral wing articulation sclerites (fig. 23).
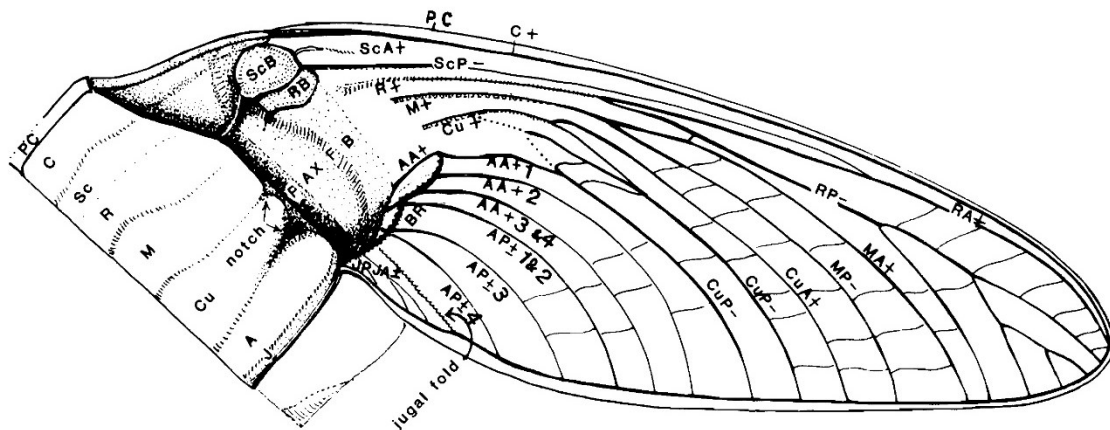

Figure 23: The interpertation of the articular region of the wing pad of *Paimbia fenestrata* specimen 2293/7 after Kukalová-Peck (1983). +, convex; -, concave; A, anal; AA, anal anterior; AP, anal posterior; Ax, axalaria; B, basivenalia; BR, brace; C, costa; Cu, cubitus; CuA, cubitus anterior; CuP, cubitus posterior; F, fulcalaria; J, jugal; JA, jugal anterior; JP, jugal posterior; M, media; MA, media anterior; MP, media posterior; P, proxalaria; PC, precosta; R, radius; RA, radius anterior; RP, radius posterior; Sc, subcosta; ScA, subcosta anterior; ScP, subcosta posterior.

The two presumed fully formed sclerites (ScB, Sc basivenale; RB, R basivenale) are in fact artefacts on the specimen 2293/7, where the cuticular surface of the wing pad is peeled off. Kukalová-Peck (1983) divided the area between the base of R vein precursor and anal brace on four columns of the supposedly fused sclerites (p, proxalaria; ax, axalaria; f, fulcalaria; b, basivenalia). However, nothing on this specimen supports the presence of these structures. Further, the author divided the attached notum into seven parts, each corresponding to one main vein precursor (PC, precosta; C, Sc, R, M, Cu and A). Again, no evidence for this assumption can be found in the specimen (fig. 24). There are two posterior transverse furrows in the notum, but they can hardly be regarded as structures connected to the presumed rows of axillary
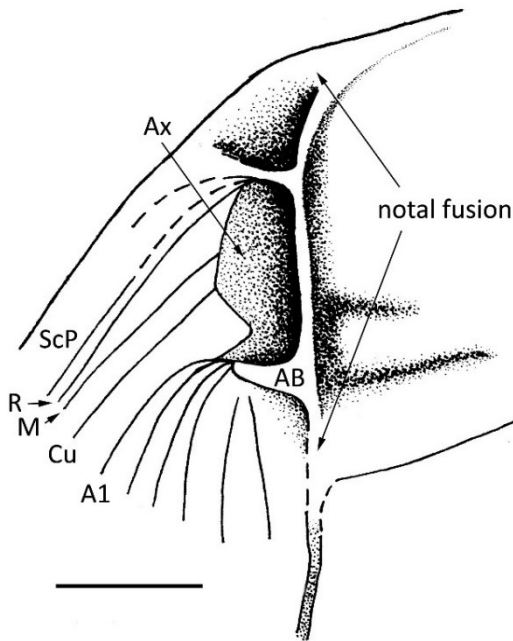
sclerites and basal sinuses of the main vein precursors.

Figure 24: The basal area of the wing pad of *Paimbia fenestrata* specimen 2293/7. AB, anal brace; Ax, axillary sclerite; A1, first anal vein; Cu, cubitus; M, media; R, radius; ScP, subcosta posterior. Scale bar = 2 mm.

Similarly, in the wing pad of *P. carpenteri* (specimen 3115/26) is interpreted by Kukalová-Peck (1983) showing the structures on the wing base as columns of articulation sclerites originated from the fusion of the ancestral sclerites. However, these seeming structures are in fact only results of artificial damage of the wing base and only one large axillary plate is discernible between the anal brace and the base of R vein precursor (fig. 25).
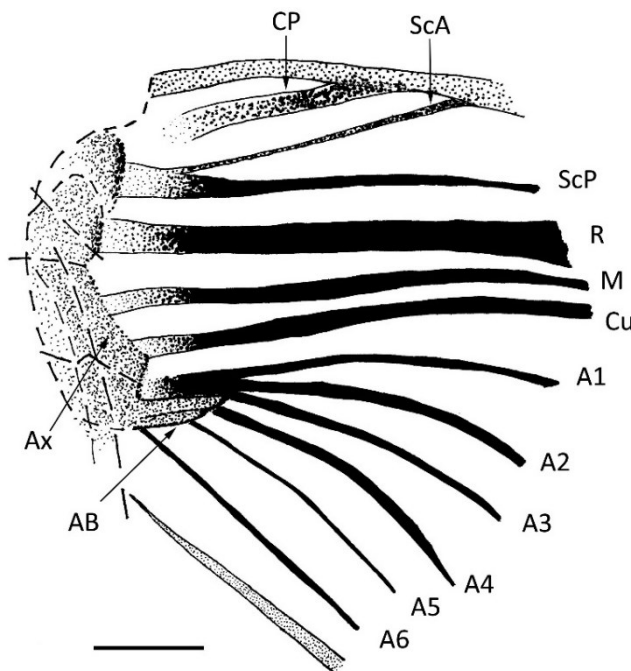
Figure 25: The basal area of the wing pad of *Parathesoneura carpenteri* specimen 3115/26. AB, anal brace; Ax, axillary plate; A1-A6, anal veins; CP, costa posterior; Cu, cubitus; M, media; R, radius; ScA, subcosta anterior; ScP, subcosta posterior; dashed line = artefacts. Scale bar = 2 mm.

5. Conclusions

Considerable variability of the patterns of wing venation and developing tracheation and lacunal channels wing pad was discovered within the series of fossil specimens assigned to the *Tchirkovaea guttata*, which suggests, that the wings and wing pads of probably more species or even genera could be included in this material. No substantial evidence was found, that would support the previous ideas about the ontogeny and wing development in Paleozoic insects (Sharov 1973; Kukalová-Peck 1976, 1978, 1983; Sinitshenkova 1979).

When compared with the developing wing pads of recent mayfly *Cloeon dipterum*, the enlarged outer margin suggest the presence of a developing wing inside cuticular case or wing sheath, therefore the fossils of the supposed larval wings could have similar structure as the wing pads of recent hemimetabolous insects. The idea that the developing wing pads changed their position to the body throughout the ontogeny is not supported by the relatively large larval specimen with small wing pads but clearly developed and markedly exposed ovipositor. Also, there is no clear evidence for the presence of subimaginal instars in the species *T. guttata* and *Paimbia fenestrata*, as the subimaginal wings show different patterns of venation and colouration than the imaginal wings.

On the other hand, evidence disputing the view of Kukalová-Peck (1983) on the ancestral wing articulation of Pterygota is presented. The series of sclerites supposedly present on the base of the larval wing pad of *Parathesoneura carpenteri* is in fact one large axillary plate fragmented by artificial damage of the wing base. Moreover, in the isolated wing pad specimen of *Paimbia fenestrata* the anterior and posterior notal fusion is discernible, supporting the more recent findings of Prokop et al. (2017a).

Another particularly interesting fact is the presence of nygma-like structures on one of the wing pads of *T. guttata*. Similar structure can be found also on the additional fossil of imago of *Paimbia ultima*. The ESEM microphotographs revealed, that the outter appearance of these structures corresponds to those found in the recent holometabolous insects.

6. Acknowledgements

7. References

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Figure S1: Wing pads of *Tchirkovaea guttata*. A, specimen 2854/2, scale bar = 4 mm; B, 3115/29, scale bar = 4 mm; C, specimen 3115/160, scale bar = 5 mm; D, specimen 3115/155, scale bar = 5 mm; E, specimen 3115/167, scale bar = 5 mm; F, specimen 3115/153, scale bar = 10 mm; G, specimen 3115/63, scale bar = 20 mm; H, specimen 3115/152, scale bar = 15 mm.
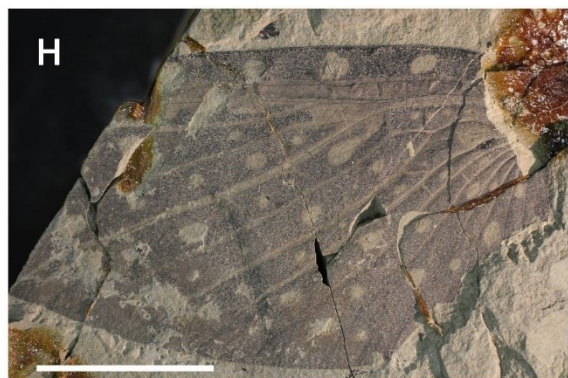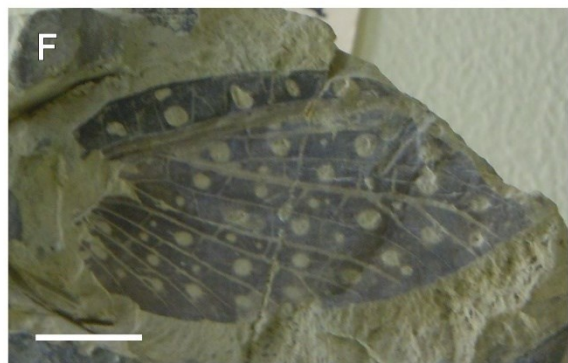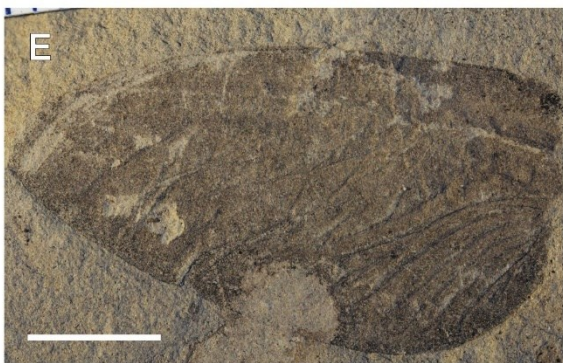
Figure S1

Figure S2: Wing pads of *Tchirkovaea guttata* (A) and *Paimbia fenestrata* (B-G). A, specimen 3115/68, scale bar = 15 mm; B, specimen 2293/6, scale bar = 15 mm; C, specimen 2293/6, scale bar = 4 mm; D, specimen 2992/1, scale bar = 10 mm; E, specimen 2293/7, scale bar = 5 mm; F, specimen 2293/4, scale bar = 20 mm.
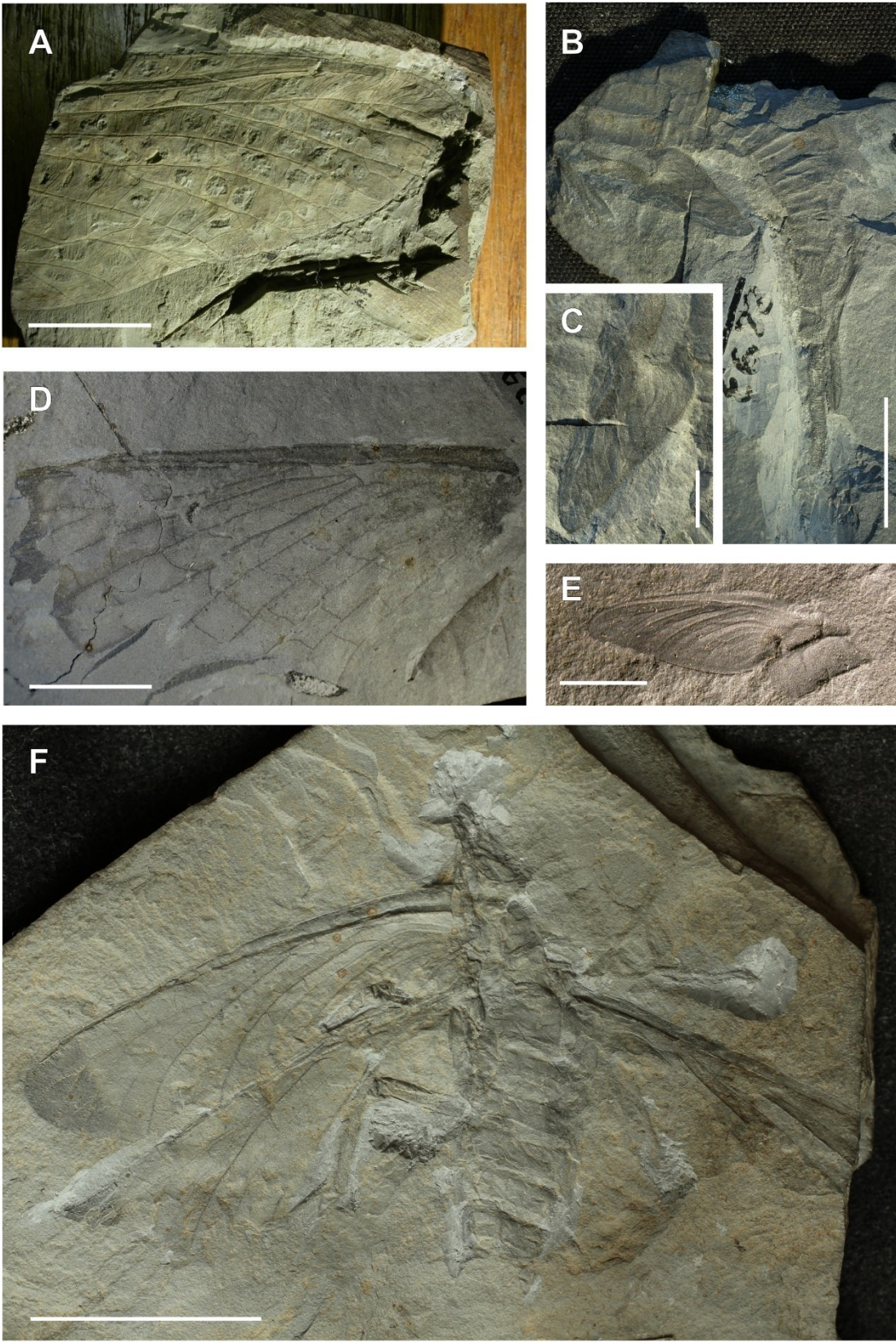
Figure S2

Figure S3: Nygmata on the wing pads of *Tchirkovaea guttata* (A) and *Paimbia ultima* (B-D). A, specimen 3115/29, scale bar = 3mm; B, C, D, specimen 3115/192, scale bar = 10 mm (B), 0,5 mm (C); C, detail of nygma from rectangle in B; D, detail of nygma from rectangle in B taken by ESEM.
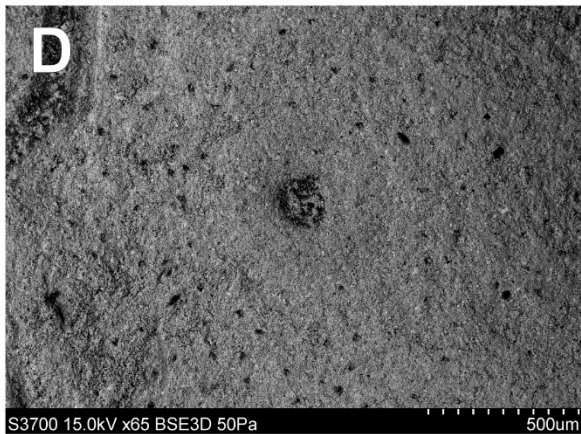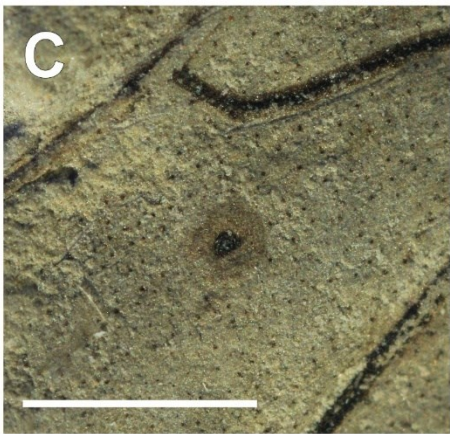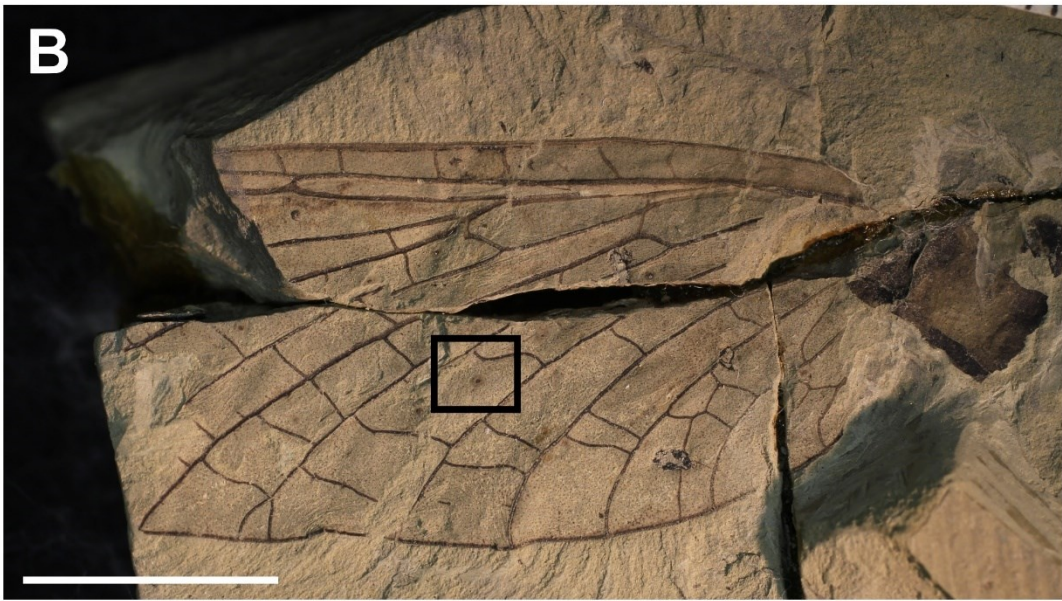
Figure S3

Figure S4: The development of wings in a recent mayfly *Cloeon dipterum* in the earliest stages. A, specimen j010, exhibiting trachea approaching the evaginations of thoracical integument on meso- and metathorax. Scale bar = 0,1 mm; B, specimen j005, showing elongation of mesothoracic wing pads. Scale bar = 0,1 mm; C, specimen j012 with visible system of lacunae and tracheae at the base of mesothoracic wing pad. Scale bar = 0,2 mm; D, specimen j017, demonstrating the development of lacunae preceding venation of adult specimens. Scale bar = 0,2 mm.
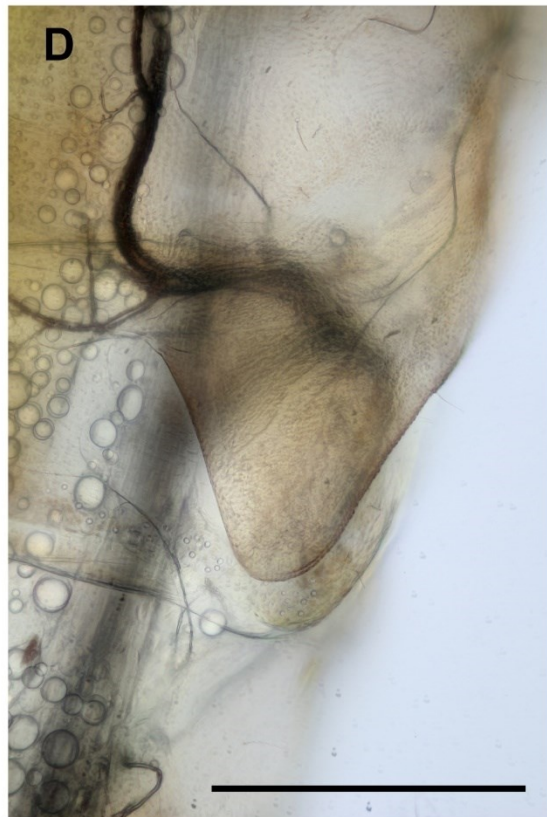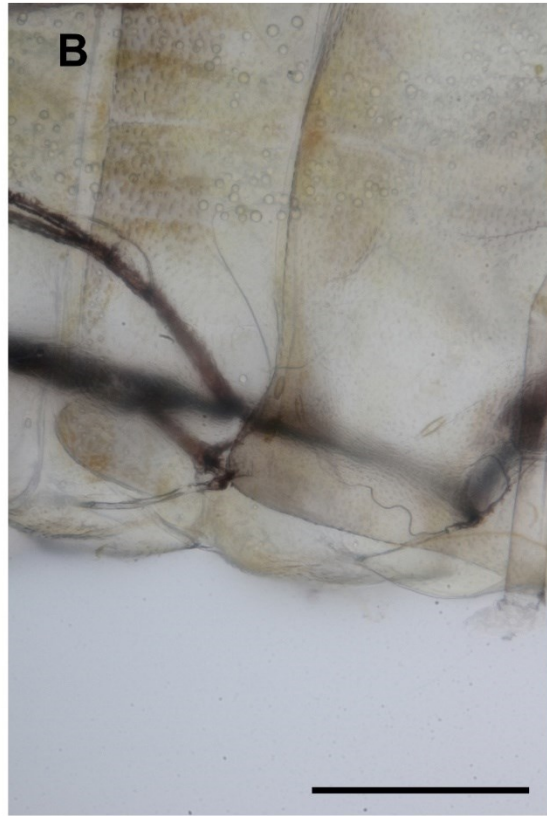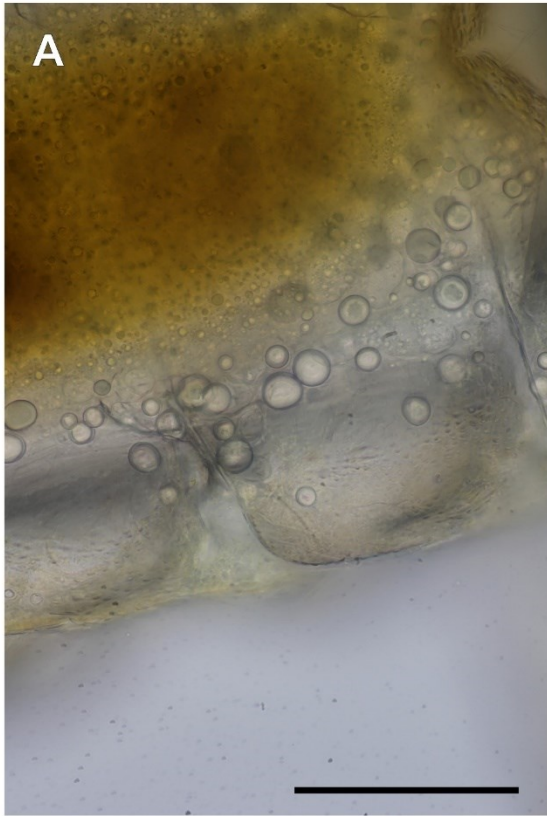
Figure S4

Figure S5: The development of wings in a recent mayfly *Cloeon dipterum* in the middle and later stages. A, specimen j016, already showing the general pattern of future venation of adult wings. Scale bar = 0,2 mm; B, specimen j019, exhibiting the development of lacunae, which are being entered by tracheae. Scale bar = 0,2 mm; C, specimen j020, cubital posterior and anal area discernible, the tracheation of the wing becomes easily recognizeable. Scale bar = 0,2 mm; D, specimen j022, the fourth branch of trachea enters the CuP (Cubitus posterior) lacuna. Scale bar = 0,2 mm.
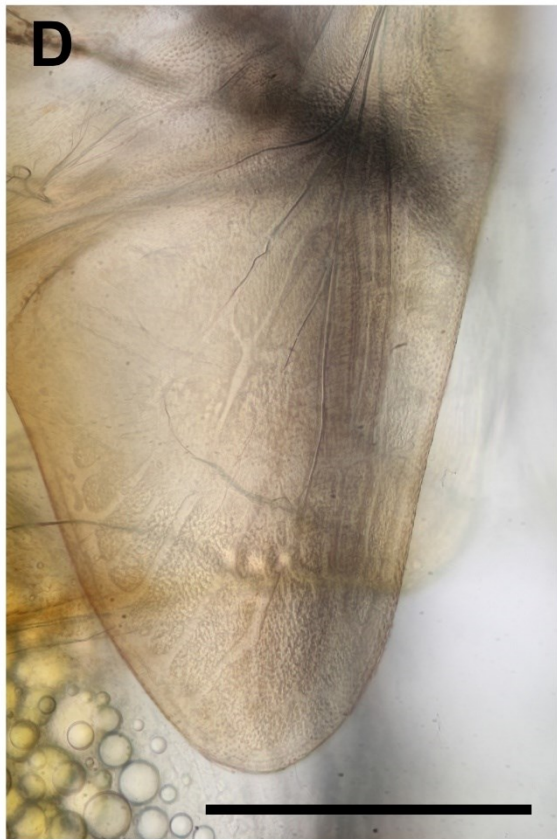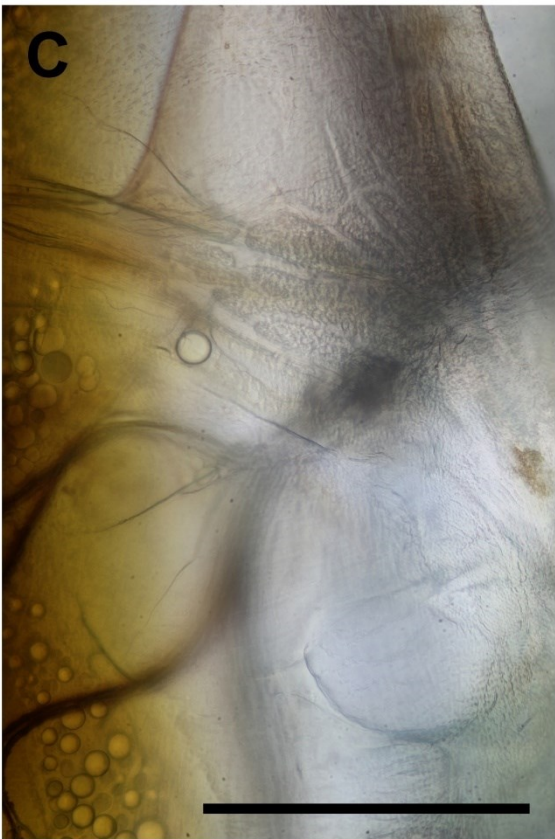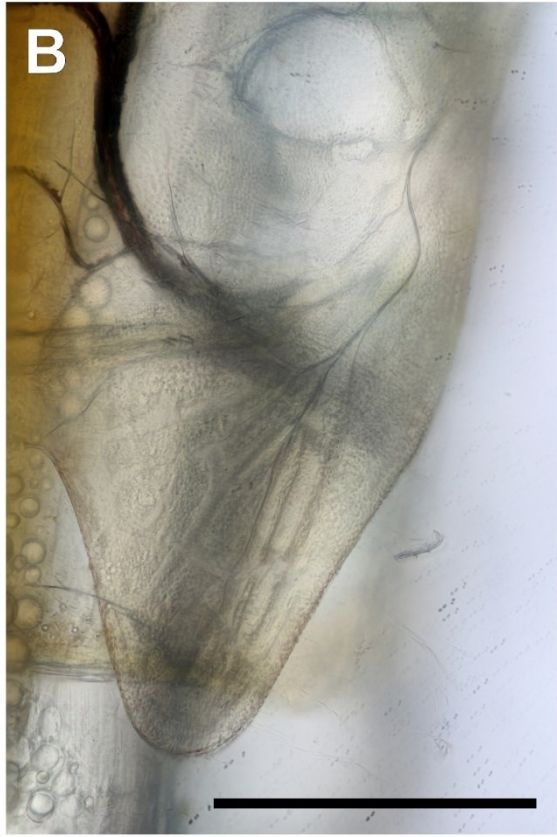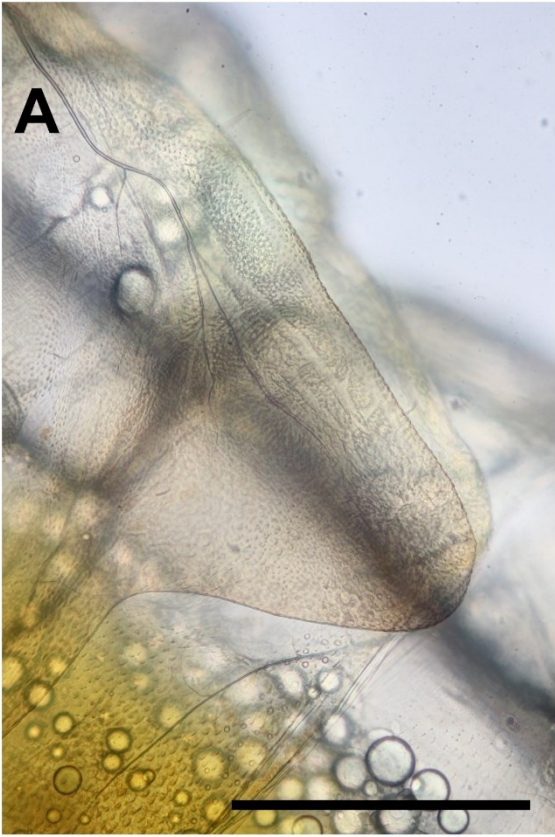
Figure S5

Figure S6: The development of wings in a recent mayfly *Cloeon dipterum* in the later larval stages and subimago. A, specimen j025, the wing pads become more elongated and the system of lacunae and tracheae is obvious. Scale bar = 0,2 mm; B, specimen j001, the last larval instar showing corrugation of the vein precursors. scale bar = 0,3 mm; C, specimen j033, subimaginal wing. Scale bar = 2 mm; D, specimen j033, subimaginal wing demonstarting a row of long setae on its posterior margin. Scale bar = 0,25 mm.

Figure S6

Table S1: An overview of the described larval stages of the order Palaeodictyoptera.

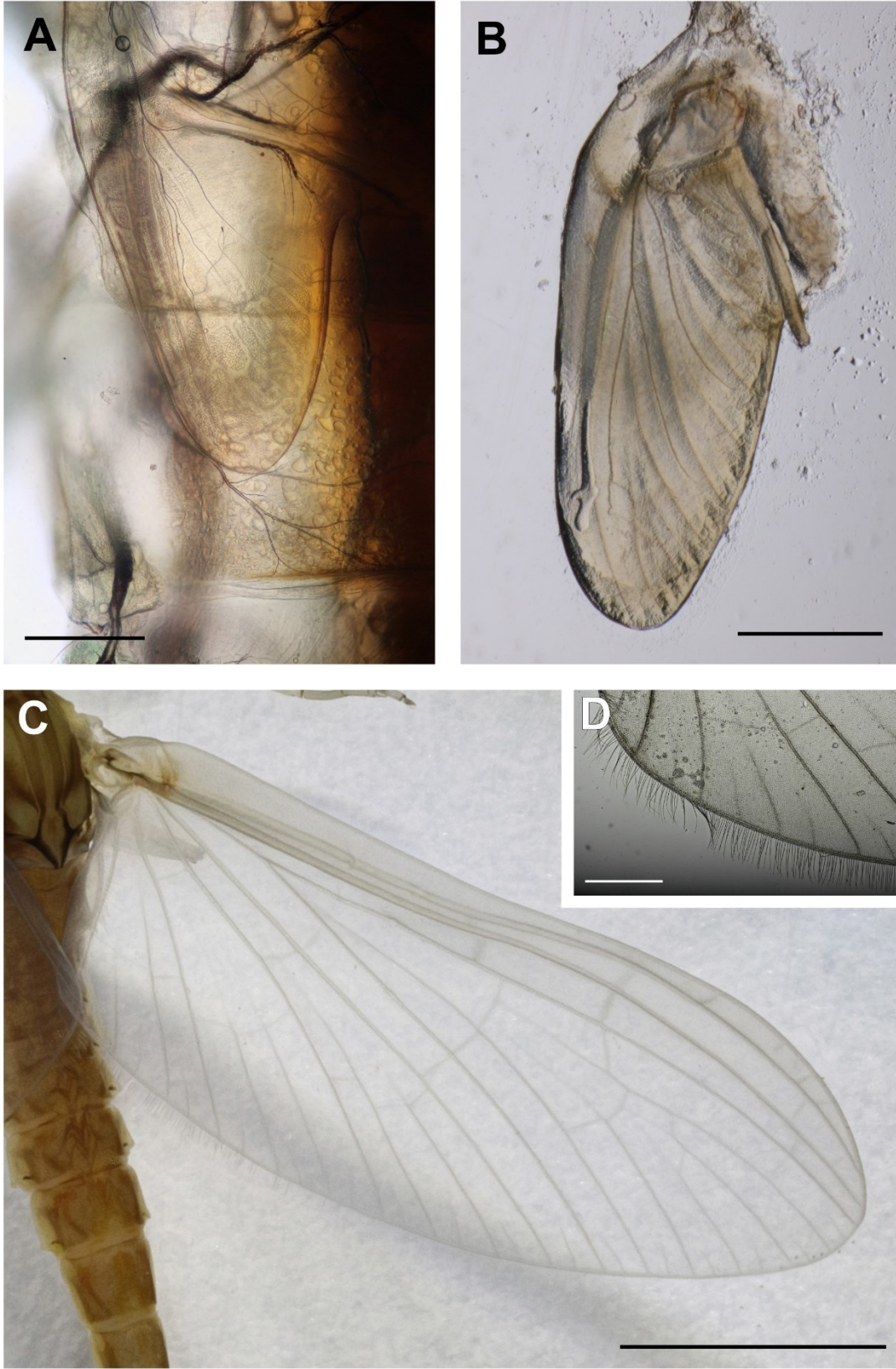| Order, family, species	Age	Locality	Reference
Breyeriidae			
Breyeria sharovi (Sinitshenkova, 1979)	Carboniferous Pennsylvanian	Paymbu River, Russia	Sinitshenkova 1979, 1981
species unknown	Carboniferous Pennsylvanian	Nors-Pas-de-Calais, France	Prokop et al. 2013
Calvertiellidae			
Moravia convergens Kukalová, 1964	Early Permian Cisuralian	Obora, Czech Republic	Kukalová-Peck & Peck 1976
Dictyoneuridae			
Goldenbergia formosa (Goldenberg, 1854)	Early Permian Cisuralian	Fat'yanikha River, Russia	Sharov & Sinitshenkova 1977
Homoiopteridae			
Adolarryia bairdi Kukalová-Peck & Richardson, 1983	Carboniferous Pennsylvanian	Mazon Creek, Illinois, USA	Kukalová-Peck & Richardson 1983
Parathesoneura carpenteri Sinitshenkova, 1977	Carboniferous Pennsylvanian	Chunya River, Russia	Sharov & Sinitshenkova 1977
Lycocercidae			
Lycodemas adolescenc Carpenter & Richardson, 1971	Carboniferous Pennsylvanian	Mazon Creek, Illinois, USA	Carpenter & Richardson 1971
Spilapteridae			
Bizzarea obscura Prokop et al., 2015	Carboniferous Pennsylvanian	Mazon Creek, Illinois, USA	Prokop et al. 2015
Vorkutoneura variabilis Sinitshenkova, 1977	Early Permian Cisuralian	Khey-Yaga River,, Russia	Sinitshenkova 2020
Tchirkovaeidae			
Paimbia fenestrata Sinitshenkova, 1979	Carboniferous Pennsylvanian	Paymbu River, Russia	Sinitshenkova 1979
Tchirkovaea guttata Zalessky, 1931	Carboniferous Pennsylvanian	North Oungua river, Russia	Zalessky 1931
	Carboniferous Pennsylvanian	Chunya River, Russia	Sinitshenkova 1979
Family unknown			
Idoptilus sp.	Carboniferous Pennsylvanian	Barnsley, Yorkshire, UK	Prokop et al. 2018
	Carboniferous Pennsylvanian	Mazon Creek, Illinois, USA	Prokop et al. 2017a
Idoptilus onisciformis Wootton, 1972	Carboniferous Pennsylvanian	Barnsley, Yorkshire, UK	Wootton 1972
Idoptilus peachii Woodward, 1887b	Carboniferous Pennsylvanian	Greenhil Quarry, Ayrshire, UK	Ross 2010
species unknown	Carboniferous Pennsylvanian	Sosnowiec, Poland	Prokop et al. 2017a

Table S2: An overview of the described larval stages of the order Megasecoptera.

Order, family, species	Age	Locality	Reference
Alectoneuridae			
Alectoneura europaea Kukalová-Peck, 1975	Early Permian Cisuralian	Obora, Moravia, Czech Republic	Kukalová-Peck 1975
Arcioneuridae			
Arcioneura juveniles Kukalová-Peck, 1975	Early Permian Cisuralian	Oborna, Moravia, Czech Republic	Kukalová-Peck 1975
Brodiidae			
Brodia priscotincta (Handlirsch, 1919)	Early Permian Cisuralian	Coseley, West Midlands, UK	Handlirsch 1919, Prokop et al. 2017
			Bolton 1921
		Tipton, West Midlands, UK	Bolton 1921
		Sedgley, West Midlands, UK	Bolton 1921
		Unknown	Bolton 1921
Lameereites sp.			Haug et al. 2016, Prokop et al. 2019
Lameereites curvipennis Handlirsch, 1911	Carboniferous Pennsylvanian	Mazon Creek, Illinois, USA	Handlirsch 1911, Prokop et al. 2019
Caulopteridae			
Cauloptera colorata Kukalová-Peck, 1975	Early Permian Cisuralian	Obora, Moravia, Czech Republic	Kukalová-Peck 1975
Mischopteridae			
Mischoptera douglassi Carpenter & Richardson, 1968	Early Permian Cisuralian	Mazon Creek, Illinois, USA	Carpenter & Richardson 1968, Prokop et al. 2019

Table S3: Revision of Tchirkovaea guttata specimens described by Sinitshenkova (1979), the specimens are sorted into putative species based on the pattern of venation.

Specimen	L	W	instar S.	F/H	Branches						Position from base			Putative species
					RP	MA	MP	CuA	CuP	1A	RA/RP	MA/MP	CuA/CuP	
2854/2	8	5	early	F	3	1	1	1	1	1	3.	2.	1.	1
3115/29	14,8	5,9	early	F	3	1	2	1	1	1	2.	3.	1.	2/4
3115/160	13,8	6,7	early	H	2+	1	2	2	2	1	3.	2.	1.	3
3115/157	14,7	7	early	H	3	1	1	1	1	1	2.	3.	1.	4/6
3115/155	19	10,6	middle	H	3	1	2	1	1	1	2.	X	1.	4
3115/167	21	11,3	middle	H	3	1	1	3	1	2	2.	2.	1.	5
3115/153	50	24	later	F	X	1	3	1	1	1	2.	3.	1.	2
3115/53	52	26	later	H	3	1	1	1	2	1	2.	3.	1.	6
3115/151	56	27	subimago	F	4	1	1	1	1	1	3.	2.	1.	1
3115/63	66	33	subimago	F	4*	1	1	X	X	1	3.	2.	1.	7
3115/152	57	27	subimago	H	X	1	2	1	1	1	3.	2.	1.	1
3115/66	70	30	imago	F	3+	1	2	1	1	1	2.	3.	(1.)	4
3115/191	70	36	imago	F	X	X	X	X	X	X	X	X	1.	4
3115/68	70	28,5	imago	H	4	1	2	1	1	2	X	X	(1.)	4
3115/193	70	X	imago	H	4	X	X	X	X	X	2.	3.	(1.)	4

L, wing pad lenght; W, wing pad width; instar S., instar assigned by Sinitshenkova; F/H, fore/hind wing pad; RP, radia posterior; MA, media anterior; MP, media posterior; CuA, cubitus anterior; CuP, cubitus posterior; 1A, first anal vein; RA/RP, point of divergence of RA and RP; MA/MP, point of divergence of MA and MP; CuA/CuP; point of divergence of CuA and CuP.