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Morfologie a evoluce vybraných skupin Palaeodictyopterida (Insecta: Palaeoptera)

Morphology and evolution of selected groups of Palaeodictyopterida (Insecta: Palaeoptera)

Disertační práce

Školitel: RNDr. Jakub Prokop, Ph.D.

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Abstrakt

Palaeodictyopterida jsou pozoruhodným nadřádem hmyzu, který tvořil značnou část diverzity hmyzu svrchního paleozoika, koncem permu však z fosilního záznamu mizí. Hlavní synapomorfií zástupců nadřádu je bodavě-sací ústní ústrojí ve formě rostra, které bylo tvořeno pěti stylety. Toto rostrum pravděpodobně sloužilo k nabodávání rostlinných pletiv a sání šťáv. Stejný typ ústního ústrojí sdílely s dospělci i larvy Palaeodictyopterida. Vnější genitálie nadřádu vykazují také důležité morfologické znaky. Samčí genitálie jsou tvořeny párem gonostylů a dvěma laloky penisu, podobnou morfologii genitálií nalézáme u recentních Ephemeroptera. Samičí genitálie Palaeodictyopterida jsou vyvinuty v kladélko, které lze srovnat s endofytickým kladélkem některých recentních Odonata. Tyto morfologické znaky také ukazují na možné postavení Palaeodictyopterida jako sesterského taxonu k Odonatoptera + Panephemeroptera.

Hlavním cílem práce bylo popsat nové zástupce řádu Megasecoptera, druhé nejpočetnější skupiny nadřádu Palaeodictyopterida. Křídelní žilnatina Megasecoptera vykazuje ve srovnání s bazálním řádem Palaeodictyoptera postupnou redukci podélných i příčných žilek. Další tělní struktury byly pozorovány hlavně u čeledí Brodiopteridae a Protohymenidae, u kterých byly nalezeny rozdíly v délce ústního ústrojí a morfologii genitálií. To nám poskytuje představu o specializaci na různé potravní zdroje v rámci jednoho řádu. Vedlejším cílem bylo studium larev řádu Palaeodictyoptera, jejichž morfologie oblasti artikulace křídelních pochev poskytla podporu pro duální teorii vzniku hmyzího křídla.

Klíčová slova: Palaeoptera, Palaeodictyopterida, srovnávací morfologie, systematika, křídelní žilnatina, svrchní paleozoikum

Abstract

Palaeodictyopterida is remarkable insect superorder, which formed a significant part of the diversity of upper Palaeozoic insects, but disappeared by the end of the Permian. The main synapomorphy of the superorder is the piercing-sucking mouthparts in the form of a rostrum consisting of five styles. This rostrum was probably used to pierce on plant tissue and for the juice sucking. The same type of mouthparts shared by adults was present also in larvae of Palaeodictyopterida. The external copulatory organs of the superorder members was also showed some morphological interests. The male genitalia consist of a pair of gonostyli and two penial lobes, similarly to the genitalia of recent Ephemeroptera. The female genitalia of Palaeodictyopterida are developed in a form of the ovipositor that can be compared with the endophytic ovipositor of some recent Odonata. This morphological features support placement of Palaeodictyopterida as sister group of Odonatoptera + Panephemeroptera.

The main aim of the work was to describe new representatives of the order Megasecoptera, the second largest group of Palaeodictyopterida. Wing venation of Megasecoptera exhibits a reduction of the longitudinal and transverse veins in comparison with the order Palaeodictyoptera. Other body structures were examined mainly in the families Brodiopteridae and Protohymenidae, where differences in the length of the mouthparts and genital morphology were found. This gives us the idea of specializing in a variety of food sources within a single order. The secondary task was to study the larvae of the order Palaeodictyoptera, whose morphology of the articulation of the wing sheats given support for the dual theory wing origin hypothesis.

Key words: Palaeoptera, Palaeodictyopterida, comparative morphology, systematics, wing venation, Late Palaeozoic

Obsah

10 Články 1–7

1 Úvod

Studium fosilního hmyzu přináší nenahraditelný zdroj informací, týkajících se evoluce a diverzity jedné z nejúspěšnějších skupin živočichů. Nejčastěji nalézanými fosíliemi hmyzu jsou jejich křídla. Podle uspořádání křídelní žilnatiny lze křídla přiřadit k jednotlivým řádům i systematicky nižším taxonům. Navzdory tomu jsou křídla ancestrálních Pterygota poměrně problematická, charakteristické znaky na nich nejsou ještě vyvinuty a nebo zde nalézáme ojedinělé znaky, jejichž srovnání s recentním hmyzem není možné. O to větší význam mají fosilní nálezy kompletních jedinců, které poskytují informace o morfologii dalších tělních struktur jako např. ústního ústrojí, artikulaci křídla a genitálií.

2 Cíle práce

Hlavním cílem práce bylo prostudovat dosud nepopsaný materiál fosilního hmyzu nadřádu Palaeodictyopterida. V první radě se jednalo o zástupce několika čeledí řádu Megasecoptera. Rozsáhlý materiál ze svrchního karbonu Číny byl použit jako podklad ke zpracování diplomové práce na téma: Morfologie a taxonomie nově objevených zástupců skupiny Megasecoptera ze svrchního karbonu severní Číny (Insecta: Palaeodictyopteroida), obhájené v roce 2013. V diplomové práci však byl hlavní důraz kladen na aplikování metod geometrické morfometrie na soubor křídel a nemohl tak být věnován dostatečný prostor ke zhodnocení ostatních tělní struktur. Zachování materiálu se později navíc ukázalo jako vhodné pro studium pomocí skenovací elektronové mikroskopie. Další dostupný materiál z permu Ruska, náležící čeledím Protohymenidae a Scytohymenidae, se taktéž vyznačoval zachováním některých dalších tělních struktur, např. ústního ústrojí. Do čeledi Brodiidae byl zařazen nový nález ze svrchního karbonu Kanady. Dalším zdrojem informací byl materiál ze sideritových konkrecí z lokality Mazon Creek (USA), z kterého byl popsán nový zástupce čeledi Protohymenidae. Stejný materiál sloužil i jako základ pro vedlejší cíl práce, kterým bylo prostudování nových i již popsaných larev Palaeodictyoptera a srovnání těchto poznatků v nových souvislostech.

3 Systematická část

Kapitola pojednává o systematice hmyzu (Insecta *sensu stricto*) s důrazem na vymřelé linie a fosilní záznam z paleozoika a mesozoika. Z důvodu poměrně úzkého tématu práce je podrobně probírána pouze skupina Palaeoptera. Morfologii a srovnání důležitých tělních struktur u skupin Panephemeroptera, Odonatoptera a Palaeodictyopterida je věnována kapitola 4.

3.1 Insecta s. str.

Taxon zahrnuje hmyz s ústním ústrojím vně hlavové kapsule (Ectognatha), neboli primárně bezkřídlé skupiny Archaeognatha, Zygentoma a křídlatý hmyz. Do nedávné doby byli Archaeognatha považováni za skupinu sesterskou k Dicondylia, která byla tvořena skupinami Zygentoma a Pterygota. Jejich apomorfií byla dikondylní mandibula, tj. mandibula, která se k hlavové kapsule připojuje na dvou místech. Naopak monokondylní mandibula byla přítomná u Archaeognatha (Wheeler a kol., 2001). Nedávná podrobná morfologická studie však tuto hypotézu vyvrátila, byla nalezena i druhá artikulace mandibuly u Archaeognatha. Dikondylní mandibula se tak stala synapomorfií Zygentoma, Pterygota a Archaeognatha (Blanke a kol., 2015a). Sesterské taxony Zygentoma a Pterygota jsou však stále poměrně dobře podpořen na základě mnoha dalších morfologických (např. Bitsch a Bitsch, 2004; Beutel a Gorb, 2006) i molekulárních studií (např. Kjer a kol., 2006; Regier a kol., 2010; Dell'Ampio a kol., 2014).

Za nejstarší doklad křídlatého hmyzu bylo ještě do nedávné doby považováno ústní ústrojí *Rhyniognatha hirsti* Tillyard, 1928 ze spodního devonu, které však podle nejnovější studie náleží spíše zástupci stonožkovců (Myriapoda) (Engel a Grimaldi, 2004; Haug a Haug, 2017). Z paleozoika (karbon–perm) pochází také nálezy náležící čeledi Dasyleptidae, jejíž zástupci byli umísťovány blízko k Archaeognatha, do vymřelé skupiny Monura (Rasnitsyn, 2000; Sharov, 1957). Později bylo toto tvrzení několika autory vyvráceno a *Dasyleptus* Brongniart, 1885 byl interpretován jako neotenická rybenka, avšak novější nálezy z triasu zařazované do téhož rodu podporují původní hypotézu (Bechly a Stockar, 2011; Montagna a kol., 2017). Archaeognatha jsou ve fosilním záznamu dále zastoupeni např. nedávno popsaným druhem *Gigamachilis triassicus* Montagna a kol., 2017 z čeledi Machilidae (Montagna a kol., 2017). Pozoruhodnost tohoto nálezu spočívá ve způsobu zachování – fosfatizaci, díky níž je možné pozorovat mimo jiné stavbu centrální nervové soustavy. Ostatní doklady tohoto řádu pocházejí z jantaru (křída, terciér), které již lze přiřadit do recentních čeledí Meinertellidae a Machilidae (např. Sturm a Machida, 2001).

Zygentoma jsou ve fosilním záznamu zastoupeni velice zřídka, nejstarší zástupce ze spodní křídy nebylo možné přesněji popsat z důvodu špatného zachování (Sturm, 1998). Další nálezy pocházejí z barmského (křída) a dominikánského (miocén–oligocén) jantaru (např. Mendes a Poinar, 2013). Z evolučního hlediska je zajímavá bazální reliktní rybenka *Tricholepidion gertschi* Wygodzinsky, 1961, která byla dříve umísťována do čeledi Lepidotrichidae. Tarsy končetin jsou pětičlánkové, zatímco u ostatních Zygentoma i Archeognatha jsou dvou- až tříčlánkové. To podporuje hypotézu, že původní tarsus byl pětičlánkový a nižší počet článků v odvozených skupinách můžeme považovat za redukce. Čeleď byla popsána na základě nálezů z baltského jantaru (eocén) a její zástupci sdílí nápadné znaky (např. velký počet abdominálních stylů a vesikul). Ty jsou ale považovány za plesiomorfie a podle Engela (2006) fosilní *Lepidothrix pilifera* Silvestri, 1912 je příbuznější ostatním rybenkám (Euzygentoma) než reliktní *Tricholepidion gertschi*.

Kontroverzní fosílie *Carbotriplura kukalovae* Kluge, 1996 ze svrchního karbonu ČR představuje podle nejnovější studie (Staniczek a kol., 2014) sesterskou skupinu křídlatého hmyzu. Původně byla přiřazena k dospělci *Bojophlebia prokopi* Kukalová-Peck, 1985 (jako bazální skupina Ephemerida, podle Kukalová-Peck, 1985), později popsána jako zástupce dnes již parafyletického taxonu Thysanura (Kluge, 1996), dále také uvažována jako bazální Zygentoma (Bitsch a Nel, 1999). Willman (2003) navrhl postavení *C. kukalovae* jako možného zástupce sesterské skupiny k Pterygota na základě výrazně zvětšených paranot na všech segmentech thoraxu. V práci Staniczka a kol. (2014) je poukázáno na podobnost laterálních výběžků abdomenu s tehdy ještě nepopsanou larvou *Bizzarea obscura*, která s jistotou patří nadřádu Palaeodictyopterida (viz **čl. 7**).

Pterygota

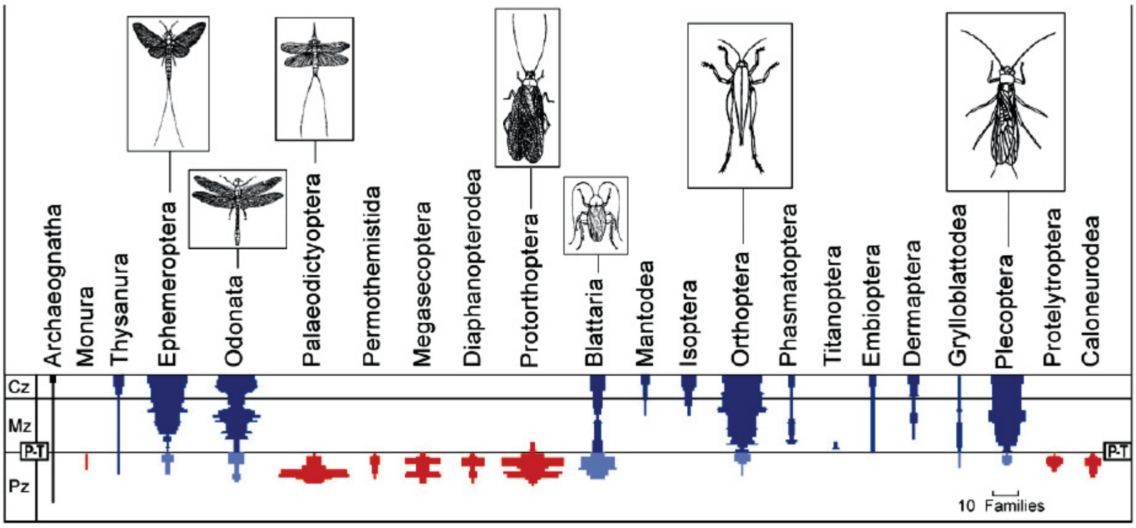
Molekulární analýzy jednoznačně podporují monofylii Pterygota (např. Kjer a kol., 2006; Misof a kol., 2007) a tím i jediný vznik křídel v rámci evoluce hmyzu (Engel a kol., 2013). Vnitřní dělění Pterygota je již problematické.

V dnešní době je uznávána monofylie taxonu Palaeoptera, který sdružuje Odonata a Ephemeroptera, sesterskou skupinu představují Neoptera. Tento koncept je potvrzován výsledky mnohých studií, ať už morfologických (např. Blanke a kol., 2012; Shear a Kukalová-Peck, 1990; Martynov, 1924; Hennig, 1969; Kukalová-Peck, 2008; Bechly 1996; Haas a Kukalová-Peck, 2001) nebo molekulárních (Wheeler a kol., 2001; Hovmöller a kol., 2002; Kjer a kol., 2006; Regier a kol., 2010). Jednou z nejdůležitějších apomorfií Neoptera je sval (t-p 13 *sensu* Matsuda, 1970), který spojuje třetí axilární sklerit s pleurou a umožňuje složení křídla v horizontální linii nad zadeček (Willkommen, 2008).

V minulosti však byly navrhovány i alternativní koncepty. Rozdíly ve výsledcích jednotlivých molekulárních i morfologických studií mohou být způsobeny předpokládanou rychlou divergencí všech tří linií (Panephemeroptera, Odonatoptera a Neoptera), které se ve fosilním záznamu najednou objevují na začátku svrchního karbonu (Carpenter, 1992; Thomas a kol, 2013). Jednou z navrhovaných možností byl koncept Chiastomyaria spojující Neoptera + Ephemeroptera s bazálními Odonata (např. Carle, 1982a, 1982b; Misof a kol., 2007; Simon a kol., 2009), nebo další koncept Metapterygota spojující naopak Neoptera + Odonata s bazálními Ephemeroptera (např. Wheeler a kol., 2001; Ogden a Whiting 2003; Willkommen a Hörnschemeyer, 2007; Beutel a Gorb, 2006).

Thomas a kol. (2013) srovnávají do té doby známé studie zabývající se základní divergencí Pterygota, zdůvodňují jejich výsledky a přiklání se ke konceptu Palaeoptera. Rozsáhlá fylogenomická studie hmyzu Misofa a kol. (2014) se také přiklání k monofilii Palaeoptera. Ke konečnému rozřešení této otázky může také přispět studium nejstarších fosílií křídlatého hmyzu.

Vznik křídla u hmyzu je považován za klíčovou událost, která se zasloužila o evoluční úspěch hmyzu a jeho diverzifikaci. Hypotézám o evoluci křídla bylo proto věnováno velké množství studií, jednotlivé teorie jsou podrobněji popsány v kapitole 4.1.1. Prvním dokladem křídlatého hmyzu je křídlo známé až ze spodního karbonu, které patří hmyzu skupiny Archaeorthoptera (Prokop a kol., 2005). Dalším nálezem ze spodního karbonu je Palaeodictyoptera *Delitzchala bitterfeldensis* Brauckmann a Schneider, 1996. Svrchní karbon je již velmi bohatý na fosílie hmyzu, nalézáme zde zástupce ancestrálních linií recentních řádů hmyzu: Archaeognatha, Zygentoma, Ephemeroptera, Odonata, Blattaria, Orthoptera, Grylloblattodea, Plecoptera, ale i řádů, které koncem paleozoika vymírají – Palaeodictyoptera, Permothemistida, Megasecoptera, Diaphanopterodea, Protorthoptera (= Archaeorthoptera), Protelytroptera, Caloneurodea (viz obr. 1; Labandeira, 2005; 2011).



Obr. 1. Diverzita vybraných řádů hmyzu vztažená k počtu známých čeledí (převzato z Labandeira, 2005); červená – řády vymírající koncem permu, modrá – ostatní řády.

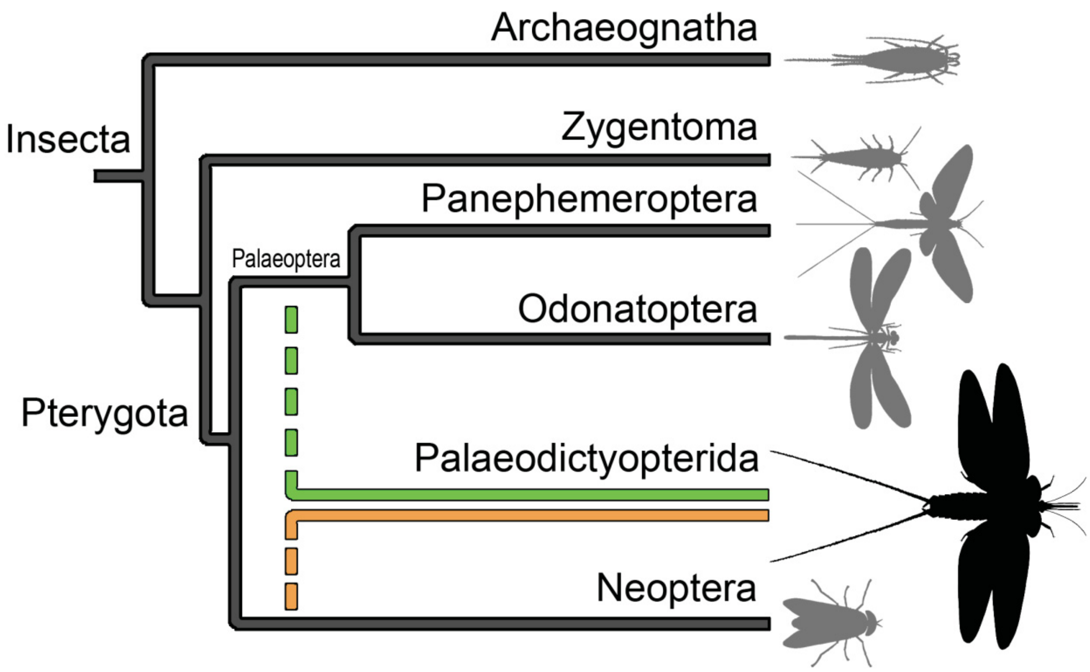
3.2 Palaeoptera

Tato skupina zahrnuje recentní Ephemeroptera + Odonata. Pokud do systému zařadíme i fosilní zástupce, potom ji tvoří Panephemeroptera + Odonatoptera (= Hydropalaeoptera) a sesterská skupina Palaeodictyopterida (např. Kukalová-Peck, 1991). Alternativní hypotézu představuje postavení Palaeodictyopterida jako sesterské k Neoptera, tvořící tak spolu skupinu Neopterygota (viz obr. 2; Sroka a kol., 2015). V této práci je upřednostněno použití nadřádu Palaeodictyopterida jako sesterského taxonu k Hydropalaeoptera. Společným znakem všech Palaeoptera je např. spojování artikulačních skleritů do větších celků, které neumožňují skládání křídel na zadeček, tak jako u Neoptera (viz kapitola 4.1.2; Kukalová-Peck, 1991).

Na bázi Hydropalaeoptera nacházíme čeleď Bojophlebiidae s jediným zástupcem *Bojophlebia prokopi* Kukalová-Peck, 1985 (Sroka a kol., 2015) ze svrchního karbonu ČR. Tento gigantický hmyz s rozpětím křídel 45 cm, byl původně popsán jako jepice. Na jeho křídle však není přítomna kostální spona, proto nemůže být zařazen do Panephemeroptera (*sensu* Staniczek a kol., 2011). Rozvětvená MP vylučuje příslušnost Bojophlebiidae k Odonatoptera, u nichž je posteriorní větev mediální žilky vždy jednoduchá (Bechly, 1996). Skupiny Panephemeroptera a Odonatoptera (= Euhydropalaeoptera *sensu* Sroka a kol., 2015) spojují vodní larvální stadia (znak považován za konvergenci) a další morfologické (např. výrazná korugace žilnatiny – střídání konvexity a konkávity sousedících žilek, přítomnost interkalárních žilek) i molekulární znaky, které byly analyzovány v mnohých studiích (viz kapitola Pterygota).

Fosilní čeleď Tintorinidae ze středního triasu nemá na rozdíl od Panephemerotpera vyvinutou kostální sponu a žilka ScP je krátká (Krzemiński a Lombardo, 2001). Z těchto důvodů je čeleď považována za Hydropalaeoptera *incertae sedis* (Staniczek, 2011).

Kontroverzní fosílie *Triplosoba pulchella* (Brongniart, 1894) ze svrchního karbonu byla po dlouhou dobu uvažována jako nejstarší bazální jepice. Prokop a Nel (2009) ji na základě basálního větvení hlavních žilek a dalších znaků přeřazují do Palaeodictyopterida. Toto umístění přehodnocuje Staniczek a kol. (2011), navrhuje obnovení taxonu Triplosobida s nejasným postavením uvnitř Palaeoptera.



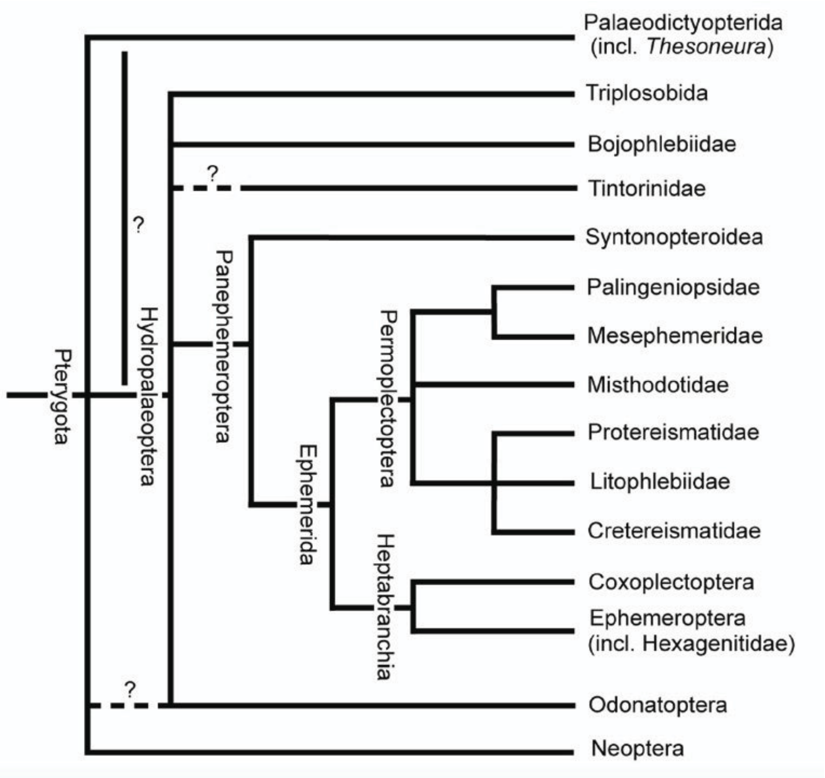
Obr. 2. Dvě hypotézy postavení skupiny Palaeodictyopterida (viz **čl. 6**, převzato a upraveno); zelená – pozice nadřádu v Palaeoptera, např. podle Kukalová-Peck, 1991; oranžová – jako sesterská skupina Neoptera, dle Sroka a kol., 2015.

3.2.1 Panephemeroptera

Synapomorfií skupiny je speciální struktura nacházející se u báze předního okraje křídla – kostální spona (Sroka a kol., 2015). Tato spona je tvořena anteriorní větví subkostální žilky (ScA), která u odvozených skupin Panephemeroptera splývá u báze s kostálním okrajem křídla, poté se výrazně odchyluje a dále pokračuje do R1 (=RA). U bazální nadčeledi Syntonopteroidea (karbon–perm) se spona nedotýká kostálního okraje, je od něj viditelně oddělena (Zhou, 2007; Prokop a kol., 2010; Staniczek a kol., 2011). Do této nadčeledi patřící zástupci rodů *Lithoneura* Carpenter, 1938 a *Miracopteron* Novokshonov, 1993 dosahují poměrně velkých rozměrů (rozpětí křídel i nad 20 cm). Permoplectoptera (perm–křída) mají kostální sponu lépe vyvinutou, řadíme sem čeledi Palingeniopsidae, Mesephemeridae, Misthodotidae, Protereismatidae, Litophlebiidae, Cretereismatidae (Staniczek a kol., 2011). Paleozoické jepice byly od zástupců recentní korunové skupiny Ephemeroptera poněkud odlišné. Měly dobře vyvinuté všechny tři páry končetin, zadní křídlo nebylo redukováno, ústní ústrojí dospělců bylo funkční (Carpenter, 1992).

Coxoplectoptera z jury a křídy sdílí s recentní korunovou skupinou Ephemeroptera uspořádání kostální spony křížící ScP a jednočlánkový tarsus s jedním drápkem. Řadíme sem pozoruhodně zachované fosilní druhy, u kterých je znám jak dospělec tak i larva. Larvy rodu *Mickoleitia* Staniczek a kol., 2011 nesou 7 párů výrazných abdominálních žaber, tracheizace křídel v křídelních pochvách je dobře rozpoznatelná (Staniczek a kol., 2011). Systematika Panephemeroptera podle Staniczka a kol. (2011) je znázorněna na obr. 3.

U recentní korunové skupiny Ephemeroptera pozorujeme u imag několik významných redukcí. Především je to postupné zmenšování zadního páru křídel, které u některých druhů vede až k jeho úplnému vymizení. Tykadla jsou velmi krátká, ústní ústrojí a tentorium redukované. Dospělec nepřijímá potravu, proto je střevo vyplněno vzduchem a slouží jako aerostatický orgán. Končetiny meso- a metathoraxu jsou zkrácené, první pár končetin napomáhá samci zachytit samici při kopulaci. Samice nemají ovipositor, samci mají penis rozdělený do dvou laloků (Bauerfeind a Soldán, 2012). Genitálie jepic budou podrobněji popsány v kapitole 4.3.



Obr. 3. Fylogenetický strom skupin Panephemeroptera (převzato ze Staniczek a kol., 2011).

3.2.2 Odonatoptera

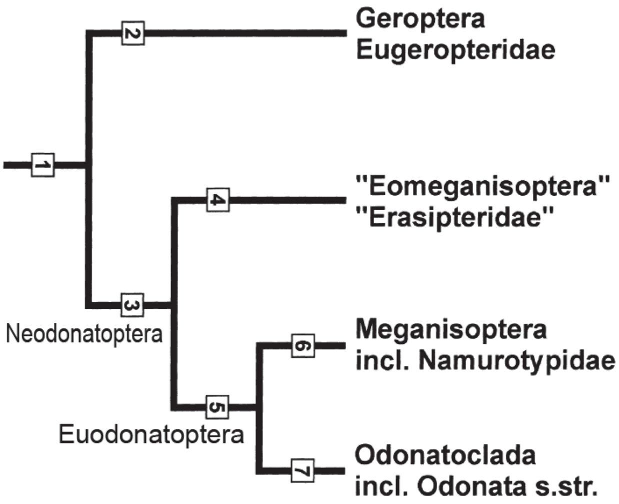
Sesterská skupina Panephemeroptera zahrnuje kromě recentních vážek (Odonata) např. i známé gigantické vážky z karbonu. Fosilní záznam Odonatoptera je však mnohem bohatší a lze díky němu sledovat vývoj znaků jak na křídelní žilnatině, tak i na dalších tělních strukturách (např. genitálie). Na křídlech lze nalézt tyto významné synapomorfie: anální pole redukované (zvláště u předního křídla), u báze se nachází anální spona tvořená propojením kubitálních a análních žilek, jednotlivé artikulační sklerity postupně splývají (Bechly a kol., 2001).

Bazální skupinou jsou Geroptera, kteří se především vyznačují vyvinutými prothorakálními křidélky se zřetelnou křídelní žilnatinou (druh *Argentinala cristinae* Petrulevičius a Gutiérrez, 2016). V nedávné studii autorů Petrulevičius a Gutiérrez (2016) je navrženo nové systematické dělení pro skupiny na bázi Odonatoptera. Původní Geroptera (*sensu* Bechly a kol., 2001) jsou rozděleni do několika nových taxonů. Pro přehlednost však zde budeme pro tyto taxony používat souhrnné označení Geroptera.

Sesterská skupina k Euodonatoptera (Meganisoptera+Odonatoclada) se nazývá Eomeganisoptera a je tvořena pouze čeledí Erasipteridae. U zástupců nacházíme dobře zachované, velmi dlouhé kladélko přesahující konec abdomenu (Bechly a kol., 2001).

Paleozoický řád Meganisoptera (Protodonata) je uvažován jako sesterská skupina k Odonatoclada. Navzájem se liší v mnoha významných znacích: v křídelní žilnatině Meganisoptera chybí diskoidální pole, nodus ani plamka nejsou vyvinuty, samci nemají sekundární genitálie (např. Nel a kol., 2009; Prokop a Nel, 2010). Skupina zahrnuje také druhy s dosud největším známým rozpětím křídel u hmyzu, přes 70 cm u *Meganeuropsis permiana* Carpenter, 1939. Avšak jsou známé i malé druhy (Tupinae). Na unikátním nálezu druhu *Namurotypus sippeli* Brauckmann a Zessin, 1989 (Namurotypidae) jsou dobře zachovány samčí genitálie, které budou podrobněji popsány v kapitole 4.3.1. Do řádu je zařazována i poměrně dobře zachovaná larva *Dragonympha srokai* Kukalová-Peck, 2009 ze sideritové konkrece svrchního karbonu (Kukalová-Peck, 2009). Zástupci této skupiny vymírají koncem permu.

Skupina Odonatoclada zahrnuje recentní korunovou skupinu Odonata a permskou čeleď Lapeyriidae, u které je již na křídle vyvinut nodus (Nel a kol., 1999).

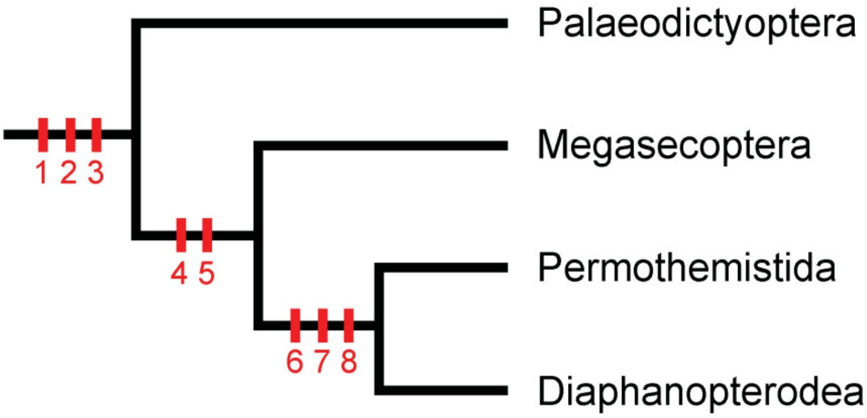


Obr. 3. Fylogenetický strom vztahů v rámci Odonatoptera (převzato Bechly a kol., 2001; zde jsou uvedené jen některé synapomorfie). **1** – viz text výše; **2** – redukce archediktyonu; **3** – RA+RP probíhají u báze těsně vedle sebe, MA spojená s RP; **4** – spolehlivé synapomorfie neznámé; **5** – redukce archediktyonu a prothorakálních křidélek; **6** – podélné žilky se přibližují k anteriornímu okraji křídla; **7** – RA+RP u báze spojené, ScP zkrácená - splývá s anteriorním okrajem.

3.2.3 Palaeodictyopterida

Sesterská skupina k Hydropalaeoptera (Odonatoptera+Panephemeroptera) – vymřelý nadřád Palaeodictyopterida zahrnuje diverzifikované zástupce herbivorního hmyzu ze svrchního paleozoika. Nejvýznamnější synapomorfií nadřádu je bodavě-sací ústní ústrojí specifické stavby, díky němuž je považován za spíše specializovanou linii křídlatého hmyzu. Ústní ústrojí bude popsáno v kapitole 4.2. V této práci je upřednostněn nejčastěji používaný systém nadřádu Palaeodictyopterida, jehož čtyřmi řády jsou: Palaeodictyoptera, Megasecoptera, Permothemistida a Diaphanopterodea (např. Shear a Kukalová-Peck, 1990).

Sroka a kol. (2015) navrhují na základě kladistické analýzy bazální postavení Palaeodictyoptera a považují Megasecoptera za sesterskou skupinu k Permothemistida + Diaphanopterodea (znaky jsou uvedené v legendě obr. 4). U bazálních Palaeodictyoptera je často vyvinuta hustá síť nepravidelně se větvících příčných žilek – archediktyon, u některých čeledí nalézáme i interkalární (vmezeřené) žilky (Carpenter, 1992). U ostatních odvozenějších řádů pozorujeme úplnou redukci archediktyonu, u některých Megasecoptera však bývá výjimečně zachován (např. čeleď Brodiidae, viz **čl. 5**).



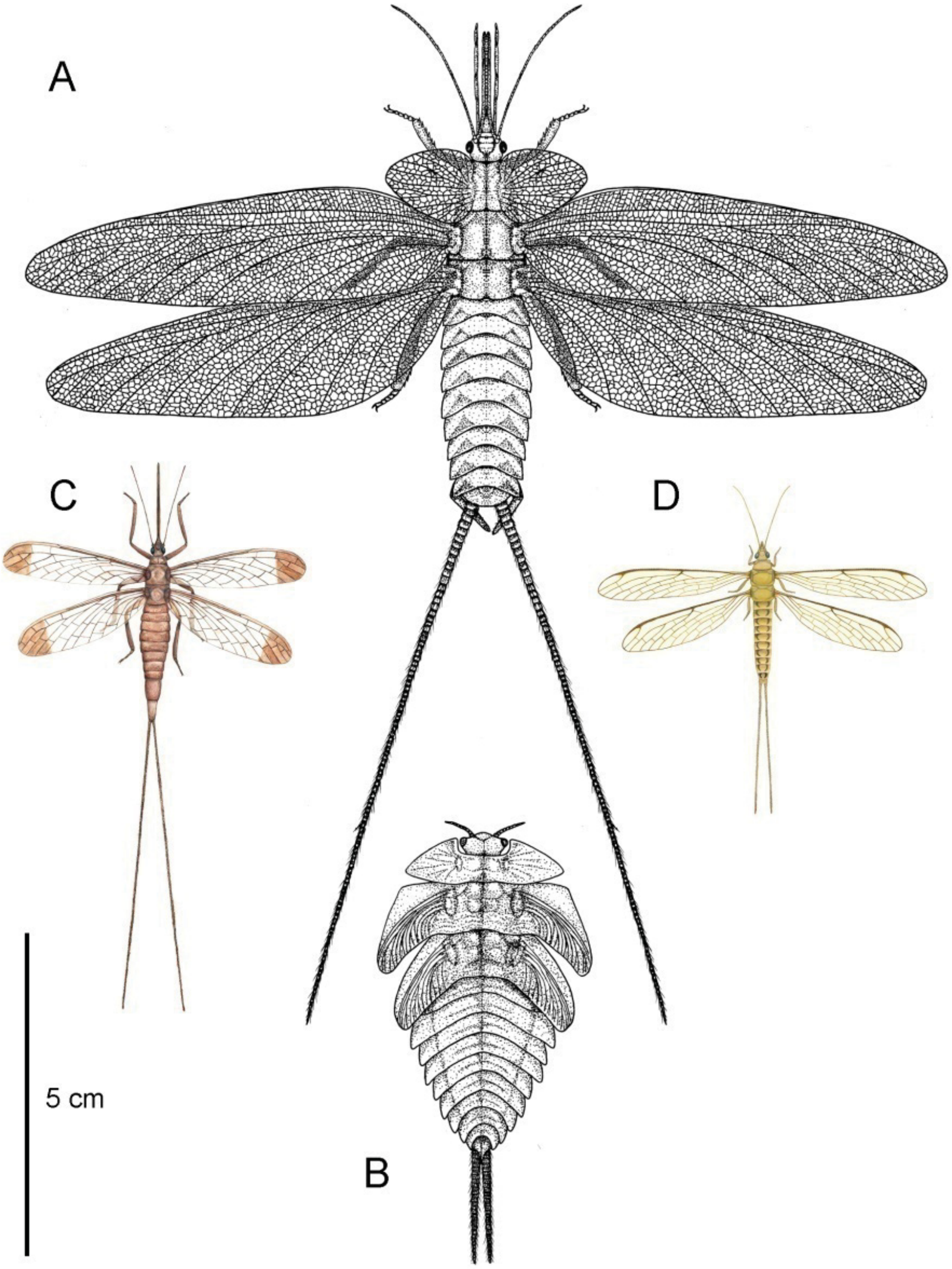
Obr. 4. Schéma nadřádu Palaeodictyopterida se společnými znaky jednotlivých řádů podle Sroka a kol., 2015. **1** – bodavě savé ústní ústrojí tvořené labiem, labrem a styletovitými mandibulami, maxilami a hypofaryngem; **2** – redukce labiálních palp; **3** – cerky dospělců delší než tělo; **4** – kostální pole předního křídla úzké; **5** – redukce archediktyonu; **6** – tři tarsální články; **7** – zvlněná ScP a RA v bazální části křídla; **8** – výrazná redukce příčných žilek.

Typy larev Palaeodictyopterida

V rámci celého nadřádu je známo pouze několik téměř nebo kompletně zachovaných larev. Oniskoidní typ robustních, tzv. trilobitových, larev s velkými prothorakálními křídelními pochvami a výraznými abdominálními laterotergity je charakteristický pro řád Palaeodictyoptera (viz obr. 5, B; Wootton, 1972). Tato silně sklerotizovaná nedospělá stadia palaeodictyopter mají rozšířené trojúhelníkovité kostální pole larválních křídel (viz **čl. 6**). V rámci řádu se zde vyskytují také protáhlé morfotypy bez trojúhelníkovitého rozšíření pole, zpravidla ale mají vždy rozšířené laterotergity abdomenu (viz **čl. 7**).

Dalším typem jsou larvy čeledi Mischopteridae (řád Megasecoptera), které postrádají rozšířené abdominální laterární tergity a místo prothorakálních výběžků mají výrazné trny, stejně jako dospělci. Larvy jsou spíše protáhlé a kostální pole křidélka není výrazně rozšířeno (viz **čl. 5**; Carpenter a Richardson, 1968).

Larvy Palaeodictyopterida sdílí s dospělci stejný typ ústního ústrojí, což naznačuje, že se živily i podobným způsobem. Většina autorů proto předpokládá terestrický způsob života larev (Carpenter a Richardson, 1968; Grimaldi a Engel, 2005). U jiných larev jsou nalézány i útvary na abdomenu interpretovány jako spirakula, které by tento terestrický způsob života podporovaly (viz **čl. 7**). Na druhou stranu však nelze na základě dostupných dat zcela vyloučit akvatický nebo semi-akvatický způsob života u larev všech zástupců nadřádu Palaeodictyopterida.



Obr. 5. Srovnání velikosti a celkového habitu některých zástupců nadřádu Palaeodictyopterida. **A**, Palaeodictyoptera *Stenodictya lobata* Brongniart, 1890 (Dictyoneuridae); **B**, Palaeodictyoptera, larva *Rochdalia parkeri* Woodward, 1913; **C**, Megasecoptera *Brodioptera sinensis* (viz **čl. 2**); **D**, Megasecoptera čeledi Protohymenidae (viz **čl. 1**).

Palaeodictyoptera

Největší řád nadřádu Palaeodictyopterida je umísťovaný na jeho bázi (viz výše). Dle Carpentera (1992) je tvořen 19 čeleděmi, do kterých však zahrnuje zástupce řádu Permothemistida. Charakteristickým znakem zástupců Palaeodictyoptera jsou křidélka na prothoraxu se zřetelnou žilnatinou. Křídla meso- a metahoraxu se k tělu připojují širokou oblastí artikulace, podélné žilky mají mnoho větví, jen zřídka navzájem splývají (viz obr. 5, A). Příčné žilky jsou někdy vyvinuté ve formě archediktyonu (např. čeleď Dictyoneuridae, Eugereonidae), ale můžou se redukovat až do jednoduché podoby (např. čeleď Spilapteridae, viz **čl. 7**). Hmyz má mohutnou stavbu těla, abdomen bývá široký s rozšířenými laterotergity. Rozpětí křídel u některých druhů, např. *Mazonopterum wolfforum* Kukalová-Peck a Richardson, 1983, může dosahovat cca 37 cm (viz **čl. 7**). Naopak některé druhy jsou malé, příkladem může být nejstarší objevená Palaeodictyoptera *Delitzschala bitterfeldensis* Brauckmann a Schneider, 1996, která dosahovala rozpětí křídel pod 3 cm.

Megasecoptera

Megasecoptera se od ostatních řádů Palaeodictyopterida odlišují téměř homonomními, protáhlými a u báze často zúženými křídly, redukovanou žilnatinou s častým spojováním podélných žilek a tendencí k přibližování žilek Sc a R k přednímu okraji křídla (tzv. kostalizace) (Kukalová-Peck, 1975). Několik autorů se věnovalo systematice uvnitř Megasecoptera. Riek (1976) vytváří určovací klíč a dělí Megasecoptera na Eumegasecoptera a Protohymenoptera, dle stupně kostalizace křídla. Podobně i Grimaldi a Engel (2005) dělí Megasecoptera na dvě skupiny, bazální Eubleptoptera a odvozené Eumegasecoptera+Protohymenoptera. Avšak čeleď Eubleptidae patří dle Carpenter (1992) spíše do řádu Palaeodictyoptera. Podle alternativního systému se Megasecoptera spojují s Permothemistida do řádu Mischopterida, který se dále člení na čtyři podřády: Eubleptina, Mischopterina, Aspidothoracina a Permothemistina (Sinitshenkova, 2002).

Permothemistida

Nejmenší řád Palaeodictyopterida – Permothemistida z permu, zahrnuje pouze dvě čeledě a to Permothemistidae a Diathemidae (Sinitshenkova, 1980). Jinými autory také nazýván Dicliptera (Grimaldi a Engel, 2005) díky redukci zadního křídla, a to buď částečné u Diathemidae nebo úplné u Permothemistidae. Řád byl popsán původně jako Archodonata Martynov 1932, později byl také považován za jeden z podřádů řádu Mischopterida (zahrnující zástupce v této práci používaných řádů Megasecoptera + Permothemistida) (Sinitshenkova, 2002).

Křídelní žilnatina se vyznačuje téměř úplnou redukcí příčných žilek, přední křídla mají výraznou plamku v apikální části mezi anteriorním okrajem křídla a RA. Tento řád, přes jedinečné znaky, s jistotou patří do nadřádu Palaeodictyopterida. Jsou totiž známí i téměř kompletní jedinci, kteří mají zachované bodavě sací ústní ústrojí, již výše uvedené jako synapomorfie nadřádu. Kromě toho zde nacházíme samčí i samičí genitálie (endofytický ovipositor) podobné stavby jako u Megasecoptera a mnohočlánkové cerky (Sinitshenkova, 1980).

Diaphanopterodea

Diaphanopterodea patří mezi menší řády Palaeodictyopterida, zahrnuje 9 čeledí (Carpenter, 1992). V minulosti byla řada zástupců tohoto řádu přiřazována k Megasecoptera. Hlavním odlišujícím znakem je schopnost skládat křídla na zadeček, podobně jako u Neoptera. Tento znak je však jen obtížně použitelný univerzálně jako synapomorfie řádu, na jeho základě totiž nelze určit izolovaná křídla (Béthoux a Nel, 2003). Dříve bylo toto specifické skládání křídel také uvažováno jako spojující znak právě s Neoptera. Později byl znak však přehodnocen a považován za konvergenci na základě podobnosti žilnatiny Diaphanopterodea s Megasecoptera a ústního ústrojí, které je synapomorfií celého nadřádu. Dalším důležitým znakem řádu Diaphanopterodea je uspořádání žilek u báze křídla, konkrétně kmeny žilek R, M a Cu probíhají těsně vedle sebe, následně se od sebe vzdalují z jednoho bodu. Bazální část křídla je popsána samostatně v kapitole artikulaci 4.1.2. Jedním ze znaků, díky němuž jsou Diaphanopterodea považováni v rámci Palaeodictyopteroida za spíše odvozenou skupinu, je tříčlánkový tarsus (Rasnitsyn a Novokshonov, 1997).

4 Morfologie vybraných struktur

4.1 Křídla

Hmyzí křídlo je bezpochyby jednou z nejpozoruhodnějších struktur v rámci členovců. Jedinečný význam má pro palaeoentomology, pro které bývá často jediným zdrojem informací. Pomocí specifického vzoru křídelní žilnatiny lze hmyz zařadit do příslušného řádu a většinou i do nižších taxonomických jednotek. Díky pevnosti a poměrně vysoké odolnosti má z hmyzího těla největší potenciál k fosilizaci.

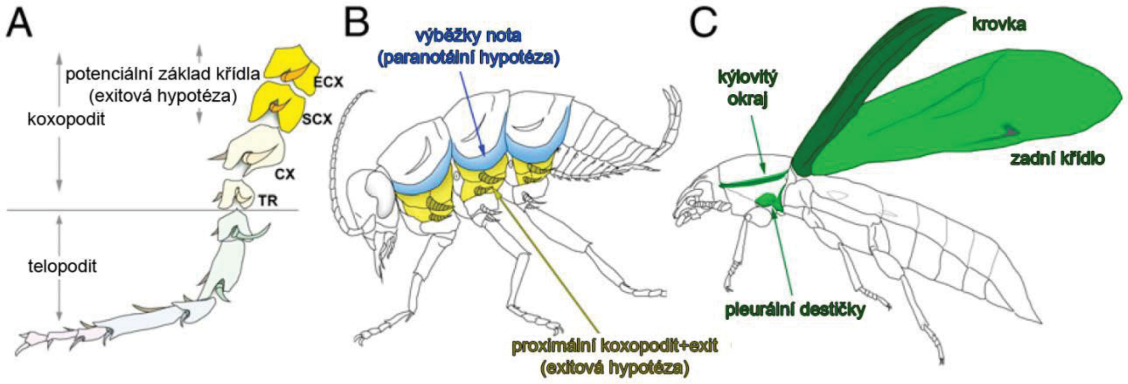
4.1.1 Hypotézy vzniku

Vznik křídel u hmyzu byl převratnou událostí, která mu umožnila obsazovat široké spektrum obývaných biotopů a dosáhnout tak velké diverzity a abundance. Diskuze o původu hmyzího křídla trvá více než jedno století a zpočátku vedla ke vzniku dvou hlavních hypotéz: paranotální a exitové. Tyto dvě hypotézy jsou podrobně popsány např. v práci Kukalové-Peck (1978).

K zastáncům paranotální hypotézy patřili např. Hamilton (1971) nebo Rasnitsyn (1981). Podle této hypotézy křídlo vzniká z laterálních výběžků dorzální části thoraxu – paranot (viz obr. 6, B). Hlavní podporou této hypotézy byl výskyt prothorakálních křidélek některých paleozoických Palaeoptera.

Podle exitové hypotézy podporované Kukalovou-Peck (1983, 1991) vznikají křídla hmyzu z exitu hypotetické části končetiny nazývané epikoxa (viz obr. 6, A, B) Epikoxa tvoří spojení mezi pleurou a dorzální částí thoraxu.

Nejnovějším konceptem vzniku hmyzího křídla je duální teorie, která propojuje původní paranotální a exitovou teorii vzniku křídla (obr. 6, C; Niwa a kol., 2010; Clark-Hachtel a kol., 2013). Byla vytvořena na základě znalostí získaných podrobným studiem vývoje křídla u octomilky *Drosophila melanogaster* Meigen, 1930, u které byl odhalen zásadní vliv genu *vestigial* na křídelní disky a přežití budoucích buněk základu křídla během larválního vývoje (Brook a kol., 1996). Dalšími pokusy na chvostnatkách a jepicích (Niwa a kol., 2010), potemnících (Clark-Hachtel a kol., 2013), plošticích (Medved a kol., 2015) a švábech (Elias-Neto a Belles, 2016) byly získány poznatky o vlivu dalších genů na vývoj křídla. Například gen *Sex combs reduced* způsobuje absenci křídla na prothoraxu. Omezením jeho působení vzniká na prothoraxu křídlu podobná struktura, která se skládá z elementů pronota i pleury (Elias-Neto a Belles, 2016).



Obr. 6. Schematické znázornění tří hypotéz vzniku křídla (upraveno a převzato z Clark-Hachtel a kol., 2013). **A**, exitová hypotéza; **B**, srovnání elementů utvářející křídlo podle paranotální (modře) a exitové hypotézy (žlutě); **C**, duální hypotéza, vyznačené elementy prothoraxu, tvořící křídla na meso- a metathoraxu.

4.1.2 Artikulační sklerity

Morfologie báze křídla hraje důležitou roli ve způsobu letu u hmyzu. Báze křídla je složena z mnoha elementů, např. axilárních skleritů, bází žilek. Skrze tento složitý systém je předávána síla thorakálních svalů vlastnímu křídlu. V minulosti se artikulačním skleritům u různých skupin hmyzu zabývala řada vědců, např. Hamilton, 1971; Brodsky, 1974, 1994; Kukalová-Peck, 1983, 1991.

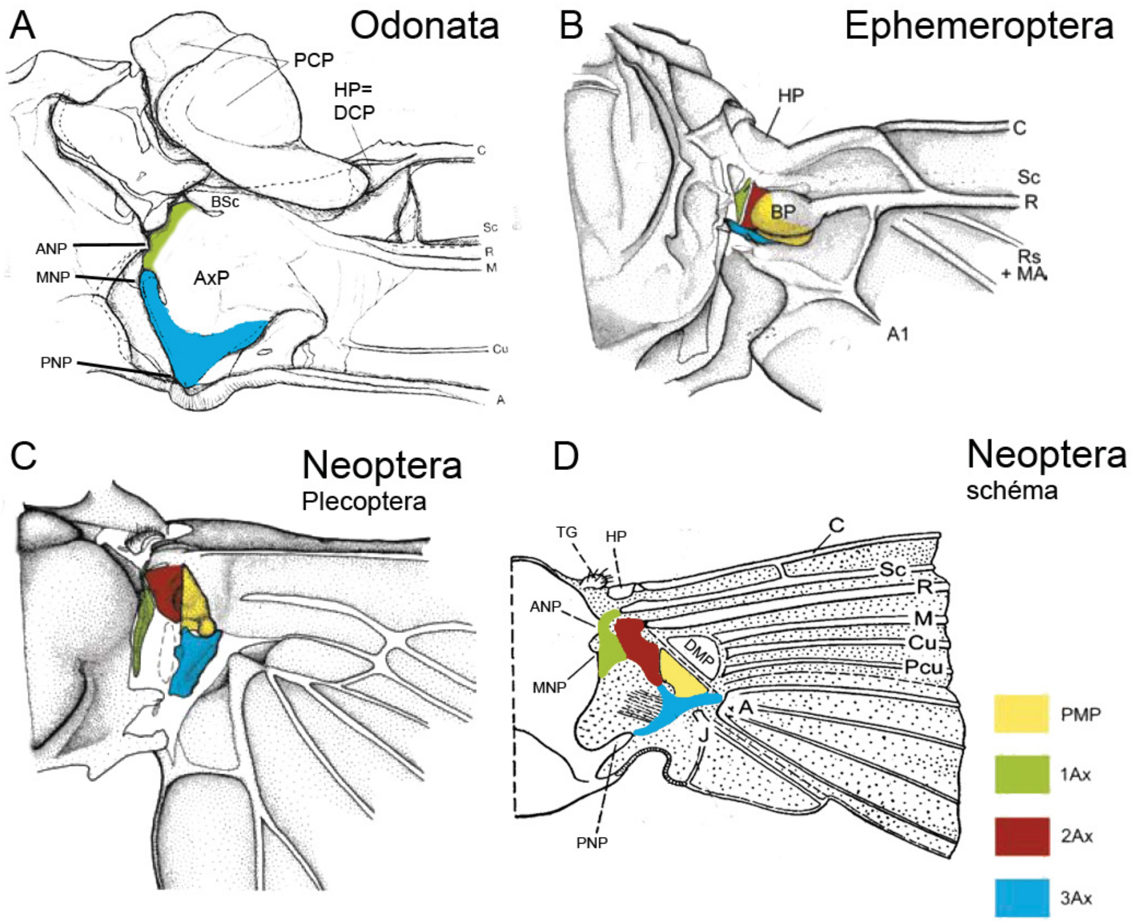
Abychom pochopili evoluci artikulace křídla, je potřeba se zaměřit na recentní i fosilní zástupce Palaeoptera. Skládací mechanismus křídel u Neoptera je totiž podstatně odlišný a je považován za odvozený (Martynov, 1924; Willkommen, 2008).

Morfologie báze křídla u Odonata byla v nedávné době revidována autory Ninomiya a Yoshizawa (2009). K interpretaci používají dva význačné body: bázi subkostální žilky (basisubcostale) a bazální sklad (oddělovací linie mezi volným křídlem a thoraxem). Na bázi křídla jsou nápadné dvě struktury, kostální destička dělící se na proximální (neboli basalare) a distální (humerální) část. Druhou, výrazně sklerotizovanou, částí je axilární destička, která se k thoraxu připojuje třemi body. Třetí menší struktura je již zmiňované basisubcostale. Autoři také vyznačují část axilární destičky, kde je předpokládána homologie s prvním a třetí axilárním skleritem jepic a Neoptera (viz obr. 7, A).

V práci Willkommen (2008) je revidována artikulace Ephemeroptera. Ta se skládá z artikulačních skleritů, které lze homologizovat se sklerity Neoptera. Na první pohled nejvýraznější strukturou je bazální destička, která vznikla pravděpodobně splynutím báze anteriorní radiální žilky, proximální mediální destičky a druhého axiláre. První axiláre se nachází proximálně od druhého axiláre (splynulého s bazální destičkou), třetí axiláre těsně přiléhá k bazální destičce, ale není s ní spojeno (viz obr. 7, B). Bazální destička jepic je pravděpodobně homologická axilární destičce vážek.

V základním plánu je báze křídla Neoptera složena ze tří axilárních skleritů (1Ax–3Ax) a dvou mediálních skleritů (proximálního a distálního). K těmto skleritům přiléhají tři výběžky nota, které artikulují s axilárními sklerity (anteriorní, mediální a posteriorní) (viz obr. 7, C, D). Ze spodní strany křídla nacházíme sklerity – anteriorně umístěné basalare a posteriorní subalare, mezi kterými vybíhá pleurální výběžek, který artikuluje s 2Ax (Snodgrass, 1935; Willkommen a Hörnschemeyer, 2007). Význačný sval (t-p 13 *sensu* Matsuda, 1970), napomáhající horizontálnímu skládání křídla, spojuje episternum s proximální částí 3Ax. U některých Neoptera je k 3Ax připojen ještě druhý sval (t-p 14), který je spojen s vrchní částí pleury (Willkommen, 2008). Modifikace artikulace u jednotlivých řádů Neoptera jsou popsány v práci Hörnschemeyer a Willkommen (2007).

V rámci nadřádu Palaeodictyopterida jsou artikulační sklerity zachovány u všech čtyř řádů. Interpretace této oblasti křídla je velmi náročná, není možno homologizovat většinu skleritů, tvoří větší splynulé destičky. Kukalová-Peck (1974b) ve své práci vyobrazuje a popisuje báze křídel několika dobře zachovaných zástupců řádů Palaeodictyoptera, Diaphanopterodea a Megasecoptera. V dalších studiích sestavuje hypotetický model ancestrální křídelní artikulace (Kukalová-Peck, 1983; Kukalová-Peck a kol., 2009). U Diaphanopterodea sklerity splývají v axilární destičku, přes kterou vede zesílená konvexní struktura, ta pravděpodobně souvisí s jedinečných mechanismem skládání křídel nad zadeček (Kukalová-Peck, 1974a, 1974b). Sinitshenkova (1980) v rámci řádu Permothemistida rozlišuje v oblasti artikulace 1.–3. axilární sklerit, mediální sklerit a tegulu.



Obr. 7. Interpretace oblasti artikulace křídla u hlavních skupin Pterygota. **A,** Odonata (převzato z Ninomiya a Yoshizawa, 2009, upraveno); **B,** Ephemeroptera (převzato z Willkommen, 2008, upraveno); **C–D,** Neoptera, **C,** Neoptera: Plecoptera (převzato z Willkommen, 2008, upraveno), **D,** Neoptera, generalizované schéma (převzato ze Snodgrass, 1935, upraveno). 1Ax, 2Ax, 3Ax – první, druhý a třetí axilární sklerit, ANP/MNP/PNP – anteriorní/mediální/posteriorní výběžek nota, AxP – axilární destička, BP – bazální destička, BSc – basisubcostale (báze subkostální žilky), HP – humerální destička, PCP/DCP – proximální/distální kostální destička, PMP/DMP – proximální/distální mediální sklerit, TG – tegula.

4.2 Hlava, tentorium a stavba ústního ústrojí

Hlava hmyzu se sestává ze 6 segmentů, které jsou seriálně homologické tělním segmentům (Posnien, 2010; Chapman, 2012). Dělí se na anteriorní procephalon, složený z okulárního, antenálního a interkalárního segmentu. Posteriorní gnathocephalon se dále člení na segmenty nesoucí ústní ústrojí: mandibulární, maxilární a labiální segment, které jsou inervovány podjícnovým gangliem. Hranice segmentů hlavy nejsou zjevně viditelné, kromě postokcipitální sutury, která odpovídá hranici mezi maxilárním a labiálním segmentem (Posnien, 2010). Existence sedmého, labrálního segment, není jistá. Jednotlivé části mozku inervují složené oči (protocerebrum), tykadla (deutocerebrum) a labrum (tritocerebrum). Vlastní ústní ústrojí hmyzu se skládá z labra (svrchní pysk), mandibul (kusadla), maxil (čelisti), labia (spodní pysk) a hypopharyngu (Chapman, 2012).

Endoskelet hlavy se nazývá tentorium, které se v generalizovaném schématu Pterygota skládá ze dvou nebo tří párů různě propojených či splynulých výběžků. Funkcí tentoria je především zpevnění hlavy a poskytnutí opory mozku a přednímu střevu. Na tentorium je upnuto mnoho svalů, např. svaly ovládající ústní ústrojí. Podle polohy rozeznáváme anteriorní, posteriorní a dorzální výběžky tentoria (Hudson, 1951). Vnitřní části výběžků splývají a dávají tak vzniknout tělu tentoria (= corpotentorium). Anteriorní výběžky tentoria se vnořují u frontoklypeálního sulku, nad anteriorní artikulací mandibul. Posteriorní výběžky jsou obecně asociovány s okcipitálním foramen, vnořují se do postokcipitálního sulku. Dorzální výběžky tentoria vybíhají z bazální části anteriorních výběžků, často ale nejsou vyvinuty vůbec (Snodgrass, 1928).

Ústní ústrojí dospělců Odonata je kousacího typu, lze tak těžko srovnávat s odvozeným ústním ústrojím Palaeodictyoptera (Blanke a kol., 2017). Imaga recentních Ephemeroptera nepřijímají potravu, proto je ústní ústrojí atrofované. Jejich larvy mají také kousací ústní ústrojí (Staniczek, 2000).

Ústní ústrojí je známo u všech řádů Palaeodictyopterida a je považováno za nejdůležitější synapomorfii tohoto nadřádu. V rámci Palaeodictyoptera jej pozorujeme u několika jedinců *Homoioptera vorhallensis* Brauckmann a Koch, 1982, dále u druhu *Eugereon boeckingi* Dohrn, 1866 (revidováno Müller, 1978), *Stenodictya laurentiauxi* Kukalová, 1970, *Lycocercus goldenbergi* Brongniart, 1885, *Monsteropterum moravicum* Kukalová-Peck, 1972 (Kukalová-Peck, 1972) a u larvy *Bizzarea obscura* (viz **čl. 7**). V práci Carpenter a Richardson (1968) je na druhu *Homaloneura dabasinskasi* Carpenter, 1964 popsán příčný řez ústním ústrojím s pěti do kruhu uspořádanými strukturami, které představují jednotlivé stylety. Rostrum *Permuralia maculata* (Kukalová-Peck a Sinichenkova, 1992) (Diaphanopterodea) je detailně popsáno v práci Kukalové-Peck (1992), později revidováno autory Rasnitsyn a Novokshonov (1997). Krátké ústní ústrojí je také známé u řádu Permothemistida, druhu *Pauciramus demoulini* Sinitshenkova, 1980 (Sinitshenkova, 1980). V rámci Megasecoptera bylo rostrum nedávno popsáno na základě několika jedinců *Brodioptera sinensis* (viz **čl. 2,3**), přesto bazální část styletů nebylo možné zrekonstruovat. Částečně zachované ústní ústrojí nacházíme také u *Sinopalaeopteryx olivieri* (čeleď Aykhalidae, viz **čl. 4**). Morfologii proximální části styletů a tentoria čeledi Protohymenidae bylo možné prostudovat a popsat na základě permského materiálu z Ruska (viz **čl. 1**). Nálezy larev Megasecoptera vykazují rostrum se zřetelnými stylety u druhů *Mischoptera douglassi* Carpenter a Richardson, 1968 (Mischopteridae) a *Lameereites curvipennis* Handlirsch, 1911 (čeleď indet.) (viz **čl. 5**; Carpenter a Richardson, 1968).

Autoři popisů se víceméně shodují na morfologii rostra Palaeodictyopterida. Svrchní pysk (labrum) je u báze široký, postupně se distálně zužuje a dosahuje přibližně poloviny délky rostra. Nejvýraznější strukturou jsou styletovité mandibuly, které jsou na okrajích silně sklerotizované a na vnitřní straně mají malé zoubky. Pod nimi se nachází pár maxil, který je opatřen dlouhými článkovanými palpami, které mírně přesahují délku styletů. Ventrálně je umístěno labium se dvěma krátkými apikálními výběžky, délkou nepřesahující mandibulární stylety (Rasnitsyn a Novokshonov, 1997). Jednotlivé stylety rostra byly drženy pevně u sebe, avšak byly samostatně pohyblivé (Kukalová-Peck, 1992; Blanke a kol., 2015b). Při porovnávání výsledků studií je zřejmé, že se ústní ústrojí v rámci nadřádu dosti lišilo, disparita tělních plánů naznačuje specializaci na různé potravní zdroje (Labandeira, 1997).

4.3 Genitálie

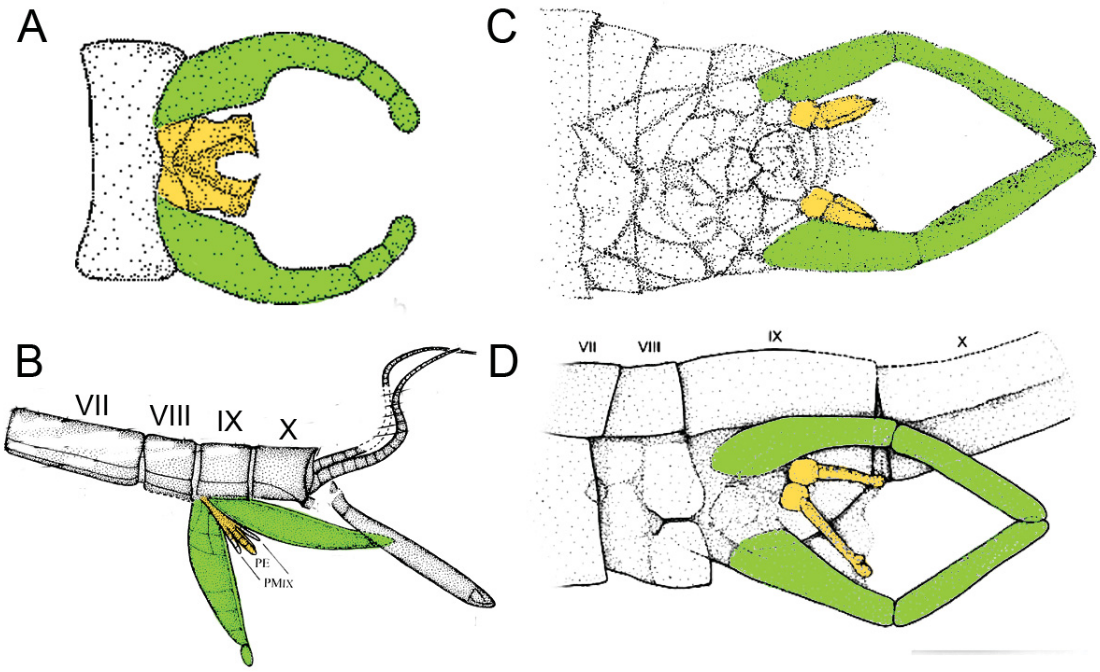
4.3.1 Samčí genitálie

Vnější samčí genitálie recentních jepic se skládají ze subgenitální destičky (=styliger, báze objímadel), která vychází z posteriorní části sterna IX. abdominálního článku. Z této destičky vycházejí obvykle několikačlánkové gonostyli (=objímadla), které při páření objímají abdomen samičky (viz obr. 8, A). Mezi nimi jsou umístěné dva laloky penisu, které jsou druhově velmi variabilní (Bauernfeind a Soldán, 2012). U některých druhů nacházíme na apexu laloků penisu části tvořící kanálek pro přenos spermií (Gaino a Rebora, 1995; Takemon 2000). Vnější genitálie permských Protereismatidae a Misthodotidae se také skládají z článkovaných gonostylů a dvou laloků penisu, jsou tak homologické genitáliím recentních jepic (viz **čl. 3**).

U recentních vážek jsou samčí genitálie vždy rozděleny do dvou celků – primárních a sekundárních genitálií. Před vlastním pářením samec přesouvá spermie z vývodu genitálií umístěného ventrálně na IX. abdominálním článku do sekundárních genitáliích, které se nacházejí na II. a III. článku. Ze sekundárních genitálií pak samec předává spermie samici (Suhling a kol., 2015). Bechly a kol. (2001) popisují ojedinělý nález vnějších samčích genitálií Meganisoptera ze svrchního karbonu u druhu *Namurotypus sippeli* Brauckmann a Zessin, 1989 (viz obr. 8, B). Oproti recentním vážkám postrádají sekundární genitálie, samčí genitálie se nacházejí pouze na IX. abdominálním článku. Skládají se z páru zploštělých gonopodů a párového penisu, který obklopují paramery IX. abdominálního článku.

Samčí genitálie nadřádu Palaeodictyopterida můžeme pozorovat v rámci řádu Palaeodictyoptera např. u druhu *Stenodictya spinosa* Brongniart 1890 (Kukalová-Peck, 1970), *Sylvohymen robustus* Martynov, 1940 (Bardohymenidae)(viz obr. 153, v Sinitshenkova, 2002), u řádu Diaphanopterodea jsou popsány u druhu *Permuralia maculata* (Kukalová-Peck a Sinichenkova 1992) (Rasnitsyn a Novokshonov, 1997). V rámci řádu Megasecoptera jsou dosud známé u čeledi Brodiopteridae a Protohymenidae (viz obr. 8, C a D; viz **čl. 1–3**).

Podle Bechly a kol. (2001) je párový penis synapomorfií Palaeoptera, což naznačují i výsledky zde předkládaných studií. Gonostyli výše zmíněných skupin jsou pravděpodobně také navzájem homologické.



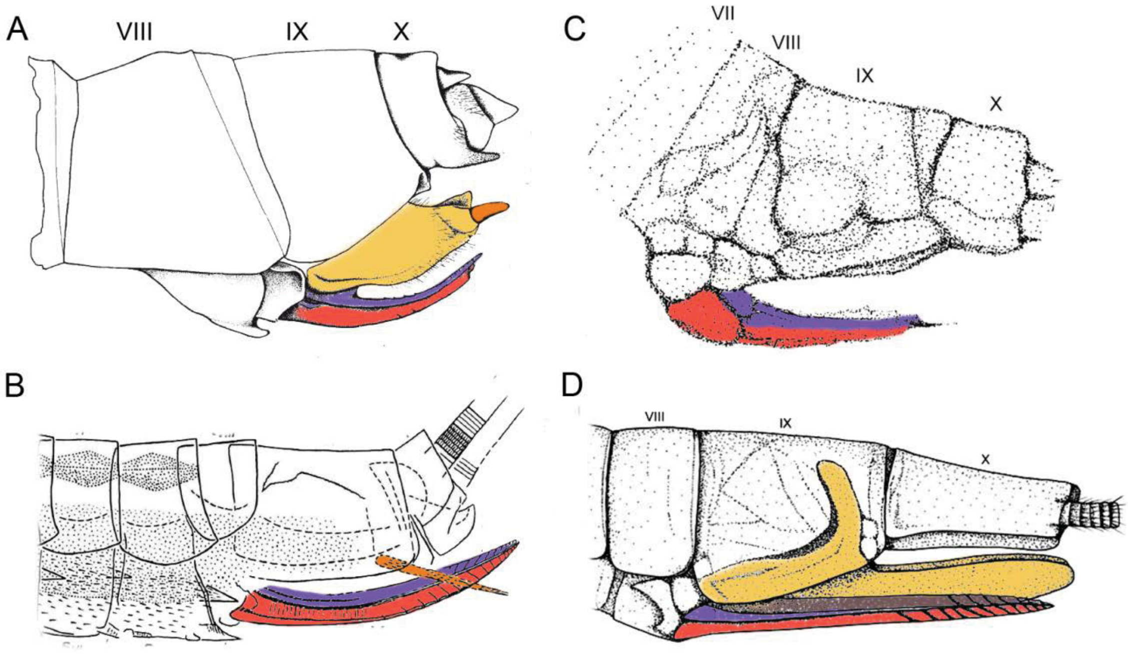
Obr. 8. Srovnání vnějších samčích genitálií u Ephemeroptera a Megasecoptera (Palaeodictyopterida). **A,** Ephemeroptera, *Austrophlebioides marchanti* Parnrong a Campbell, 1997 (převzato z Parnrong a Campbell, 1997, upraveno); **B,** Odonatoptera, Meganisoptera, *Namurotypus sippeli* Brauckmann a Zessin, 1989 (převzato z Bechly a kol., 2001, upraveno); **C–D,** Megasecoptera, **C,** Protohymenidae: *Permohymen schucherti* (viz **čl. 1**, upraveno); **D,** Brodiopteridae: *Brodioptera sinensis* (viz čl. **2, 3**, upraveno); VII–X – články abdomenu, zeleně – gonostyli (objímadla), žlutě – laloky penisu.

4.3.2 Samičí genitálie

Vnější samičí genitálie hmyzu jsou v základním plánu složené ze sternálních elementů VIII. a IX. abdominálního článku, jejichž gonapofýzy tvoří vlastní ovipositor u skupin hmyzu, kde je kladélko vyvinuto. Genitální otvor je zpravidla umístěn mezi bázemi gonapofýz VIII. segmentu (Snodgrass, 1931). Výjimku tvoří recentní Ephemeroptera, kde jsou vnější samičí genitální struktury velmi variabilní, např. se ovidukty spojují ve společný vývod, nebo ústí na povrch dvěma samostatnými gonopory, které se nacházejí v intersegmentální membráně mezi VII. a VIII. abdominálním článkem (Bauernfeind a Soldán, 2012).

Samičí genitálie některých recentních vážek, konkrétně zástupců Zygoptera a většiny Aeshnidae (Anisoptera), mají formu endofytického kladélka. To se skládá ze tří párů valvul. První a druhý pár valvul jsou výběžky VIII. a IX. sternitu, které jsou opatřeny zoubky sloužící k nařezávání rostlinných pletiv a vytváření otvorů pro nakladení vajíček. Funkce třetího páru valvul je chránit ovipositor a také jsou na něm vyvinuty styly, které napomáhají k nalezení vhodného substrátu pro nakladení vajíček a regulují pozici vajíček (viz obr. 9, A, Matushkina a Gorb, 2002; Matushkina, 2008; Suhling a kol., 2015). Z fosilních nálezů nelze opomenout ovipositor *Erasipteroides valentini* (Brauckmann, 1985), který však postrádá valvuly krycí a valvuly V1 a V2 jsou mírně zahnuté a prodloužené, výrazně přesahují konec zadečku (Bechly a kol., 2001).

Kladélko, podobající se endofytickému kladélku recentních Odonata, nacházíme u nadřádu Palaeodictyopterida. Zástupci řádu Diaphanopterodea mají kladélko dlouhé a mírně zahnuté, stylus je tenký, protáhlý (viz obr. 9, B; Rasnitsyn a Novokshonov, 1997). Ovipositor podobného tvaru nalézáme i u Permothemistida (Sinitshenkova, 1980). U Megasecoptera nacházíme dlouhý, téměř rovný ovipositor s nápadnými krycími valvulami bez stylu (Brodiopteridae, viz **čl. 2** a **3**; obr. 9, D) nebo krátký a mírně zahnutý (Protohymenidae, viz **čl. 1**; obr. 9, C; také u Foririidae, Béthoux a kol., 2004).



Obr. 9. Srovnání vnějších samičích genitálií u Odonata a Palaeodictyopterida. **A,** Odonata, *Epiophlebia superstes* (Selys, 1889) (převzato z Matushkina, 2008, upraveno); **B–D,** Palaeodictyopterida, **B,** Diaphanopterodea: Parelmoidae: *Permuralia maculata* (Kukalová-Peck a Sinichenkova 1992) (převzato z Rasnitsyn a Novokshonov, 1997, upraveno); **C,** Megasecoptera: Protohymenidae: *Permohymen schucherti* (viz čl. **1,** upraveno); **D,** Megasecoptera: Brodiopteridae: *Brodioptera sinensis* (viz čl. **2,3**, upraveno); VII–X – články abdomenu, červeně – první pár valvul ovipositoru, fialově – druhý pár valvul, oranžově – třetí, nepravé (krycí) valvuly se stylem vyznačeným tmavším odstínem.

5 Metodika

5.1 Dokumentace vzorků

Stereomikroskop + fotografie

Pro pozorování fosilních vzorků byl primárně používán stereomikroskop Nikon SMZ 645 a Leica MZ 12.5. Kresby byly vytvořeny buď pomocí Leica MZ 12.5 s nástavcem *camera lucida* (umožňující přímé kreslení při současném pozorování vzorku) nebo ze složených fotografií se souběžnou korekcí kresby se vzorkem pod stereomikroskopem. Fotografie vzorků byli pořízeny digitálním fotoaparátem Canon D550 s objektivy MP-E 65 mm a EF 50 mm, následně složeny v programu Helicon Focus Pro a poté popřípadě upraveny v Adobe Photoshop CS6. Některé vzorky byly před fotografováním pokryty vrstvou ethanolu pro zvýraznění struktur.

Digitální mikroskop

Digitální mikroskop Keyence VHX VH-Z20UR umístěný na paleontologickém oddělení Národního muzea byl využit k vytvoření 3D modelů, např. detailů thoraxu larev (viz **čl. 6**). Výhodou modelů je možnost úprav v softwaru VHX-5000 communication software a pozdější využití v dalších studiích.

ESEM

Skenovací elektronový mikroskop (SEM) je dnes velmi rozšířený přístroj pro studování povrchu nejrůznějších materiálů. Jeho oblíbenost v minulosti významně narostla po jeho zdokonalení na environmentální skenovací elektronový mikroskop (ESEM), který byl uveden v roce 1988 (Joy, 2006; Danilatos, 2009). Pro pozorování vzorků pomocí SEM je nutné, aby byl vzorek zbaven vody, jejíž evaporace by ve vakuu by způsobila smrštění a znehodnocení vzorku. Pokud vzorek není dobrým elektrickým vodičem, je nezbytné ho opatřit vrstvou kovu, obvykle 3–10 nm silnou vrstvou zlata (Joy, 2006). Tento proces u ESEM však odpadá, vzorek je pozorován při nízkém tlaku vzduchu v komoře (kolem 50–70 kPa), proto nepotřebuje být zcela zbaven vody. Také není zapotřebí pozlacení, které u fosilního materiálu často není možné. Pomocí ESEM byly v rámci výzkumu pozorovány vzorky ze sideritových konkrecí i kompresní fosílie (viz **čl. 1, 3, 6 a 7**) Nejlepších detailů bylo dosaženo u materiálu z Číny, pravděpodobně díky dobré vodivosti horniny, obsahující hodně uhlíku a také díky jemnosti sedimentu (viz **čl. 3**). Snímky byly pořízeny pomocí ESEM Hitachi S-3700N na paleontologickém oddělení Národního muzea a poté upraveny pomocí Adobe Photoshop CS6

Rentgenová počítačová mikrotomografie + 3D modelování

Data pro vytvoření 3D modelu pomocí mikro-CT byly získány ve Středoevropském technologickém institutu CEITEC v Brně na přístroji GE phoenix|X-ray tomography system v|tome|x. Samotný 3D model hmyzu ze sideritové konkrece byl vytvořen za pomoci softwaru Amira. Výsledky byly prezentovány v **čl. 6** a příloze **čl. 7**, dostupné online.

5.2 Materiál

V této kapitole jsou shrnuty informace o fosilním materiálu z několika lokalit, na jehož základě byla vypracována stěžejní část studií.

Permská lokalita Tshekarda (kungur) se nachází na levém břehu řeky Sylva, západním svahu pohoří Ural v Rusku. Fosílie se zde nacházejí v jílovcích souvrství Koshelevka (Ponomaryova a kol., 1998). Materiál z Tshekardy byl popsán v **čl. 1**.

Početný materiál ze svrchního karbonu Číny (lokalita Xiaheyan, autonomní oblast Ningxia) pochází ze souvrství Tupo, z baškiru (ekvivalent namuru B–C, stáří cca 315 Ma). Hmyz je zde zachován v podobě kompresních fosílií v nejvýše položené jednotce souvrství Tupo, ve vrstvách šedočerných a černých břidlic (Zhang a kol., 2012). Tento materiál tvořil základ pro **čl. 2, 3** a **4**.

Další zkoumaný materiál pochází ze sideritových konkrecí svrchního karbonu lokality Mazon Creek (Illinois, USA), která je oprávněně považována za *Lagerstätte*. Tímto termínem se označují naleziště s unikátním způsobem zachování fosílií živočichů i rostlin. Zachování v sideritových konkrecích umožňuje oproti kompresním fosíliím studovat reliéf, fosílie je zachována víceméně trojrozměrně (Wittry, 2012). Je proto možné tyto je zkoumat pomocí mikro-CT. Materiál z Mazon Creek byl využit ve studiích **1, 5, 6** a **7**.

Materiál popsaný v **čl. 5** pochází z Fosilních útesů Joggins (= Joggins Fossil Cliffs, souvrství Joggins, pennsylvan, Cumberlandská pánev, Nova Scotia, Kanada), které patří k světovému dědictví UNESCO.

Ostatní materiál, pocházející z dalších lokalit karbonu a permu, sloužil primárně pro srovnání s nově popisovaným materiálem a jeho původ je podrobně popsán v jednotlivých studiích.

6 Výsledky

V rámci práce byli v první řadě studovaní zástupci pěti čeledí řádu Megasecoptera. Menší, avšak neméně důležitou součástí práce, bylo studium larev řádu Palaeodictyoptera.

V rámci původně permské čeledě **Protohymenidae** byl přidán nový rod a druh, rozšiřující výskyt čeledě do svrchního karbonu. Dále byl popsán nový druh rodu *Protohymen*, který zachováním báze ústního ústrojí a tentoria významně přispívá k poznání dosud neznámé morfologie těchto struktur. Příbuzná čeleď **Scytohymenidae** byla doplněná o nového jedince druhu *Scytohymen extremus*, který jako první v rámci čeledě ukazuje morfologii tělních struktur včetně detailů jednotlivých facet složených očí (viz **článek 1** ve formě submitovaného manuskriptu).

Studium materiálu ze svrchního karbonu Číny doplnilo poznatky o čeledích **Brodiopteridae** a **Aykhalidae**. Nový druh *Brodioptera sinensis* (**Brodiopteridae**) umožnil vedle studia rozsáhlého souboru křídel také zhodnocení jedinečných tělních struktur jako např. extrémně dlouhého ústního ústrojí a samčích i samičích genitálií (viz **článek 2**). Pomocí environmentálního skenovacího elektronového mikroskopu byly odhaleny mikrostruktury na povrchu těla *B. sinensis* (viz **článek 3**). Čeleď **Aykhalidae** byla rozšířena o dva nové rody (druhy *Sinopalaeopteryx olivieri*, *S. splendens* a *Namuroptera minuta*) a morfologii ústního ústrojí (viz **článek 4**).

Nový druh čeledi **Brodiidae** ze svrchního karbonu, *Brodia jogginsensis*, jako první v rámci čeledě ukazuje pozici všech křídel. Práce je také rozšířena o diskuzi týkající se některých larev, které by mohly patřit zástupcům této čeledě (viz **článek 5**).

Studie založená na larválních stadiích řádu **Palaeodictyoptera** může posloužit jako podpora nejnovější teorie duálního původu křídla z elementů pronota i pleury (viz **článek 6**). Na několika larvách je představena morfologie báze křídelní pochvy, kde je zřetelně oddělená anteriorní artikulovaná část (původem z pleurálních elementů) a posteriorní část, plynule propojená se skutelem nota (původem z nota).

Poslední článek se věnuje zástupcům čeledi **Spilapteridae** (řádu Palaeodictyoptera) ze svrchního karbonu. Vedle popisu nového jedince *Homaloneura* cf. *dabasinskasi* je zde popsána jedinečně zachovaná larva *Bizzarea obscura*, která s jistou pravděpodobností náleží do stejné čeledě. Tato larva se morfologicky výrazně odlišuje od dosud popsaných larev Palaeodictyoptera (viz **článek 7**).

7 Poděkování

Tímto bych ráda poděkovala všem, kteří mi umožnili studium a následnou realizaci výzkumu. Zvláště bych chtěla poděkovat spoluautorům předkládaných článků a institucím za možnost půjčení materiálu k podrobnému studiu. Dále nesmím opomenout vyjádřit díky všem kolegům a přátelům z biologické i geologické sekce Přírodovědecké fakulty Univerzity Karlovy, z paleontologického a entomologického oddělení Národního muzea, jakožto i ostatním lidem mimo tyto instituce, kteří mi byli důležitou oporou. Výzkum byl finančně podpořen těmito granty: GAUK č. 596213 (2013–2015), GAČR č. 14-03847J (2014–2016).

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9 Shrnutí článků

V následující přehledu jsou stručně shrnuty obsahy článků předkládaných jako stěžejní část disertační práce. Pro přehlednost jsou rozděleny do dvou tematicky jednotných celků, přičemž první se zaměřuje výhradně na řád Megasecoptera. Druhá část se věnuje řádu Palaeodictyoptera, konkrétně studiu larev jako zdroje jedinečných informací týkající se ontogeneze křídel a zároveň jejich evolučního významu.

9.1 Část 1: Megasecoptera

První článek tvoří ucelenou studii čeledí Protohymenidae a Scytohymenidae včetně deskripce dosud nepopsaných taxonů z lokalit v Rusku a USA. Další tři články se zabývají podrobným popisem početného materiálu ze svrchního karbonu Číny. Jde o nové zástupce čeledí Brodiopteridae a Aykhalidae. Pátý článek se týká čeledi Brodiidae, taktéž je zde popsán nový druh z naleziště v Kanadě. Přínos těchto pěti studií spočívá nejen v popisu nových taxonů, ale především v komplexním srovnání různých, u Megasecoptera dosud neznámých, tělních struktur.

Článek 1

Pecharová, M. & Prokop, J. (submitovaný manuskript). Morphology, function and evolution of megasecopteran families Protohymenidae and Scytohymenidae of the Late Palaeozoic.

Článek se zabývá do této doby nepopsaným materiálem z USA (svrchní karbon) a Ruska (perm) náležící do čeledí Protohymenidae a Scytohymenidae. Doplňuje poznatky o dosud neznámých tělních strukturách u Megasecoptera jako jsou endoskelet hlavy (tentorium) nebo jednotlivé facety složeného oka a navrhuje upravenou interpretaci křídelní žilnatiny u obou čeledí.

Článek 2

Pecharová, M., Ren, D. & Prokop, J. 2015. A new palaeodictyopteroid (Megasecoptera: Brodiopteridae) from the Early Pennsylvanian of northern China reveals unique morphological traits and intra-specific variability. *Alcheringa* 39: 236–249.

Objev unikátního materiálu ze svrchního karbonu Číny umožnil popis a podrobné prozkoumání nového druhu *Brodioptera sinensis* (Megasecoptera), patřící do čeledi Brodiopteridae. Je to vůbec první zástupce Megasecoptera z lokality Xiaheyan (autonomní oblast Ningxia, Čína). Tuto takzvanou Qilianshanskou entomofaunu dosud tvořili zástupci těchto skupin: Palaeodictyoptera, Odonatoptera, stem-Grylloblattodea, stem-Dictyoptera, stem-Orthoptera, stem-Plecoptera. Někteří zástupci Archaeorthoptera a Grylloblattodea jsou zastoupeni několika desítkami jedinců. Stejně tak i *Brodioptera sinensis* byla popsána na základě 54 jedinců, což přináší možnost studia obou pohlaví, pozorování vnitrodruhových rozdílů v žilnatině křídla a celkovou rekonstrukci habitu.

Ústní ústrojí odpovídá stavbě bodavě-sacího ústního ústrojí již popsaného na dalších zástupcích Palaeodictyopterida, ale je zajímavé svojí délkou, která dosahuje téměř třetiny délky těla. Nitkovitá tykadla mírně přesahují ústní ústrojí, končetiny jsou tenké s dlouhým femurem a tibií. Samčí genitálie tvoří nápadné gonopody (objímadla) a oddělené dva laloky penisu, nápadně se podobající genitáliím recentních Ephemeroptera. Vnější samičí genitálie tvoří kladélko s pilovitými apikálními částmi prvních a druhých valvul, třetí valvula je prodloužená a nemá stylus. Morfologii kladélka lze srovnávat s kladélkem endofytického typu u některých druhů recentních vážek.

Variabilita křídel spočívá v různém počtu větví RP, jednoduché nebo rozdvojené A1 a různě umístěných příčných žilek. Zajímavější je ale výskyt několika jedinců bez zbarvených konců křídel, kteří mají méně výraznou žilmatinu. Je tak možné, že jde o čerstvě svlečené jedince, kde ještě není sklerotizace dokončená a nebo jde o nedospělé jedince (odpovídající subimagu jepic).

Vedle popisu nového druhu je zde navrženo přeřazení druhu *Sylvohymen pintoi* z lokality Hagen-Vorhalle v Německu (čeleď Bardohymenidae) právě do čeledi Brodiopteridae, jako *Brodioptera pintoi*. Také je zde diskutován blízká příbuznost čeledi Xenopteraidae s Brodiopteridae, hlavně na základě většího počtu análních žilek, které vybíhají z báze křídla. Tento typ análního pole je v rámci Megasecoptera méně častý.

Článek 3

Prokop, J., Pecharová, M. & Ren, D. 2016. Hidden surface microstructures on Carboniferous insect *Brodioptera sinensis* (Megasecoptera) enlighten functional morphology and sensorial perception. *Scientific Reports* 6: 28316;
doi: 10.1038/srep28316.

V článku jsou rozšířeny poznatky z **čl. 2**, kde byl popsán druh *Brodioptera sinensis.* Způsob zachování tohoto hmyzu v černých břidlicích umožnil pozorování materiálu pomocí environmentální skenovací elektronové mikroskopie. Byly pozorovány sety na povrchu celého těla, zvláště pak na ústním ústrojí, membráně křídel, zadečku, samčích i samičích genitálií a cerkách. Došlo k upřesnění interpretace morfologie samčích i samičích vnějších genitálií.

Článek 4

Pecharová, M., Prokop, J. & Ren, D. 2015. Early Pennsylvanian aykhalids from Xiaheyan of northern China and their palaeogeographical significance (Insecta: Megasecoptera). *Comptes Rendus Palevol* 14: 613–624.

Článek se v první řadě zaměřuje na popis dalších zástupců Megasecoptera z lokality Xiaheyan (svrchní karbon Číny) a navazuje tak na **čl. 2**. Tentokrát jde o čeleď Aykhalidae se dvěma novými rody *Sinopalaeopteryx* a *Namuroptera*, které jsou popsány hlavně na základě křídelní žilnatiny. U druhu *Sinopalaeopteryx olivieri* navíc nacházíme i ústní ústrojí, *Namuroptera minuta* se zase vyznačuje velmi dlouhými tykadly a zachováním všech křídel. Další část článku se věnuje hlavním znakům v žilnatině Megasecoptera a srovnáním Aykhalidae s příbuznou čeledí Sphecopteridae.

Článek 5

Prokop, J., Pecharová, M., Nel, A., Grey, M. & Hörnschemeyer, T. 2017. A remarkable insect from the Pennsylvanian of the Joggins Formation in Nova Scotia, Canada: insights into unusual venation of Brodiidae and nymphs of Megasecoptera. *Journal of Systematic Palaeontology*; doi: 10.1080/14772019.2017.1283364.

Článek pojednává o zástupcích čeledi Brodiidae, kteří se vyznačují značnou velikostí a výrazně stopkovitými křídly. Zde popsaný druh, *Brodia jogginsensis*, je prvním dospělým jedincem zástupcem této čeledi, kde jsou zachována všechna křídla v původní pozici (rozpětí křídel kolem 11 cm), avšak morfologii těla není možné z důvodů horšího zachování podrobněji popsat. Nález je významný také svojí lokalitou, jímž jsou Fosilní útesy Joggins (= Joggins Fossil Cliffs, souvrství Joggins, pennsylvan, Cumberlandská pánev, Nova Scotia, Kanada), které patří k světovému dědictví UNESCO. V práci jsou taktéž zveřejněny fotografie dalších zástupců čeledě Brodiidae.

Další část se věnuje larvám přiřazeným do této čeledě i jiným larvám Megasecoptera.

9.2 Část 2: Vývoj křídel u Palaeodictyoptera

Studie doplňují poznatky o larválních stadiích řádu Paleodictyoptera, navrhují novou interpretaci různých struktur, především křídelních pochev a jejich artikulace.

Článek 6

Prokop, J., Pecharová, M., Nel, A., Hörnschemeyer, T., Krzemińska, E., Krzemiński, W. & Engel, M. S. 2017. Paleozoic nymphal wing pads support dual model of insect wing origins. *Current Biology* 27: 263–269.

Cílem této studie bylo poskytnout podporu pro duální hypotézu vzniku křídla pomocí studia larev řádu Palaeodictyoptera. Byl využit dosud nepopsaný materiál z konkrecí svrchního karbonu Polska, ale i popsané larvy *Idoptilus onisciformis* Wootton, 1972 a *Rochdalia parkeri* Woodward, 1913 ze svrchního karbonu UK. Další materiál pochází ze sideritových konkrecí svrchního karbonu Lagerstätte Mazon Creek (Illinois, USA) – jednotlivě zachované křídelní pochvy, které byly zařazeny do rodů *Idoptilus* Wootton, 1972 a *Lycodemas* Carpenter a Richardson, 1971. V oblasti artikulace křídelních pochev, které jsou zachované v propojení s thoraxem (např. u *I. onisciformis*) je zřetelně oddělená anteriorní artikulovaná část (původem z pleurálních elementů) a posteriorní část, plynule propojená se skutelem nota (původem z nota). Ke studiu larev byly využity moderní metody, jako např. skenovací elektronová mikroskopie, digitální mikroskopie a vytváření 3D modelů pomocí rentgenové počítačové mikrotomografie.

Článek 7

Prokop, J., Nel, A., Engel, M. S., Pecharová, M. & Hörnschemeyer, T. 2016. New Carboniferous fossils of Spilapteridae enlighten postembryonic wing development in Palaeodictyoptera. *Systematic Entomology* 41: 178–190.

Základem pro studii byl materiál ze sideritových konkrecí svrchního karbonu Lagerstätte Mazon Creek (Illinois, USA). V první řadě byl popsán další jedinec druhu *Homaloneura cf. dabasinskasi* (čeleď Spilapteridae).

Do stejné čeledi Spilapteridae pravděpodobně patří, stejně jako výše zmíněná *H. dabasinskasi,* i cca 7 cm dlouhá larva *Bizzarea obscura.* Na základě ústního ústrojí, velkých prothorakálních i abdominálních laterálních výběžků a robustních mnohočlánkových cerků je larva přiřazena do řádu Palaeodictyoptera, s čeledí Spilapteridae ji spojují abdominální články příčně symetricky rozčleněné prostřednictvím dvou výrazných rýh. Larva má poměrně dobře vyvinuté kladélko, což ukazuje na pokročilé larvální stadium, na druhou stranu má poměrně malé křídelní pochvy. Tento fakt by tak vyloučil dříve zastávanou hypotézu o postupném graduálním růstu křídel v průběhu ontogeneze. Výrazně vyvinutá abdominální spirakula *B. obscura* podporují spíše terestrický způsob života larvy.

10 Články

Školitel a zároveň korespondenční autor všech zde prezentovaných již publikovaných článků tímto prohlašuje, že souhlasí s uvedeným podílem jednotlivých autorů na těchto publikacích.

RNDr. Jakub Prokop, Ph.D.

Článek 1

Pecharová, M. & Prokop, J. (submitovaný manuskript). Morphology, function and evolution of megasecopteran families Protohymenidae and Scytohymenidae of the Late Palaeozoic.

Podíl autorů: MP: korespondenční autor, texty: úvodní kapitola, popisy, interpretace jednotlivých struktur, diskuze, studium materiálu, dokumentace, obrazové tabule, kresby, rekonstrukce; **JP**: texty: abstrakt, diskuze, shrnutí, úpravy textů.

BMC Evolutionary Biology

Morphology, function and evolution of megasecopteran families Protohymenidae and Scytohymenidae of the Late Palaeozoic

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Abstract:	Background. Megasecoptera is extinct group of insects bearing specialized rostrum-like mouthparts as synapomorphy for all members of the Late Paleozoic Palaeodictyopterida and remarkably petiolate wings unable to flex backward. The placement of family Protohymenidae was unclear in the beginning, initially regarded as a close relative of Hymenoptera but currently supporting the position among Megasecoptera. Although the majority of megasecopteran species were examined by conventional microscopy techniques during the twentieth century, the morphology details and supposed function of keystone structures remains poorly studied. Results. Here we demonstrate close up morphology of Protohymenidae and Scytohymenidae uncovering new insights into head with endoskeleton as tentorium, mouthpart structures with discernable proximal part of stylets controlled by muscles, surface of compound eyes consisted of hexagonal pattern of large facets, structure and microstructure on wings, and reconstruction of male and female external genitalia by use of ESEM and light stereomicroscopy tools. Furthermore, we describe Protohymen novokshonovi sp. n. based on exceptionally preserved fossil from early Permian of Tshekarda in Russia showing the crucial details and the earliest species of Protohymenidae as Carbohymen testai gen. et sp. n. from the late Carboniferous siderite nodule of Mazon Creek in Illinois, USA. Conclusions. Our comparative study confirmed the set of structural and microstructural details on wings like composite anterior wing margin, development of apical cell and external genitalia previously unknown. Based on the obtained results and comparison of homologous structures known primarily on extant relatives as mayflies and dragonflies we outlined for the first time the function for mouthpart stylets, structure of tentorium, vision provided by large hexagonal ommatidia and male copulatory structures bearing curved claspers to hold female during copulation and penial lobes with seminal grooves.	
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Morphology, function and evolution of megasecopteran families Protohymenidae and Scytohymenidae of the Late Palaeozoic

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Background. Megasecoptera is extinct group of insects bearing specialized rostrum-like mouthparts as synapomorphy for all members of the Late Paleozoic Palaeodictyopterida and remarkably petiolate wings unable to flex backward. The placement of family Protohymenidae was unclear in the beginning, initially regarded as a close relative of Hymenoptera but currently supporting the position among Megasecoptera. Although the majority of megasecopteran species were examined by conventional microscopy techniques during the twentieth century, the morphology details and supposed function of keystone structures remains poorly studied.

Results. Here we demonstrate close up morphology of Protohymenidae and Scytohymenidae uncovering new insights into head with endoskeleton as tentorium, mouthpart structures with discernable proximal part of stylets controlled by muscles, surface of compound eyes consisted of hexagonal pattern of large facets, structure and microstructure on wings, and reconstruction of male and female external genitalia by use of ESEM and light stereomicroscopy tools. Furthermore, we describe *Protohymen novokshonovi* sp. n. based on exceptionally preserved fossil from early Permian of Tshekarda in Russia showing the crucial details and the earliest species of Protohymenidae as *Carbohymen testai* gen. *et* sp. n. from the late Carboniferous siderite nodule of Mazon Creek in Illinois, USA.

Conclusions. Our comparative study confirmed the set of structural and microstructural details on wings like composite anterior wing margin, development of apical cell and external genitalia previously unknown. Based on the obtained results and comparison of homologous structures known primarily on extant relatives as mayflies and dragonflies we outlined for the first time the function for mouthpart stylets, structure of tentorium, vision provided by large hexagonal ommatidia and male copulatory structures bearing curved claspers to hold female during copulation and penial lobes with seminal grooves.

Keywords

Insecta, Palaeodictyopterida, Megasecoptera, caput, tentorium, ommatidium, wing venation, external genitalia, Carboniferous, Permian.

Background

Palaeodictyopterida is group comprising four extinct insect orders: Palaeodictyoptera, Permothemistida, Diaphanopterodea and herein studied Megasecoptera. These orders share unique piercing and sucking mouthparts as synapomorphy with discernable elongated mandibulary and maxillary stylets and prominent maxillary palps [1, 2].

Megasecoptera comprise 24 families, albeit many of them were originally described only on the basis of isolated wings (e.g. Brodiidae) [3–7]. However, the megasecopteran morphology of other body structures was recently clarified in number of families. For instance, a discovery of *Brodioptera sinensis* Pecharová, Ren & Prokop, 2015 revealed, besides the wings, the structure of mouthparts and external genitalia, and also the cuticular microstructures as setae on wing membrane or hook like ridges on the first two pairs of valvules of endophytic ovipositor by the application of ESEM [8, 9].

Megasecopteran family Protohymenidae was the first time considered as a separate order Protohymenoptera with putatively shared traits in wing venation that indicate ancestral relationship to Hymenoptera [10]. This hypothesis was solely based on the wing venation of several species from the Lower Permian of Kansas. Martynov [11] doubted the assignment of order Protohymenoptera as ancestral to Hymenoptera (Holometabola) and suggested the placement among Palaeoptera, close to Megasecoptera. Carpenter [12] examined a large collection of Protohymenidae including few specimens with body structures from Elmo (Kansas, U.S.A.). He followed the placement of Protohymenidae close to Megasecoptera and suggested a new interpretation of the wing venation considering the polarity of the main longitudinal veins. Martynov [13] retained to use of separate order Protohymenoptera and established another family Scytohymenidae, with a monotypic genus *Scytohymen*. Carpenter [14] transferred Protohymenidae into Megasecoptera (and used Protohymenoptera as suborder) doubting the main diagnostic veinal character proposed by Martynov.

All species assigned to Protohymenidae are up to now known from the Permian localities in Russian Federation (Archangelsk region, Perm district) and United States of America (Kansas and Oklahoma) which were situated on Laurussia. The putative member *Sunohymen xishanensis* Hong, 1985 from the Lower Permian Shanxi Formation in China (originally supposed to be of Carboniferous age [15, 16]) should be excluded from Protohymenidae and transferred to Bardohymenidae due to the corresponding venation pattern.

In this paper we present body structures including endoskeleton of head, mouthparts with stylets and muscle connectives, compound eyes with hexagonal pattern of ommatidia, detailed morphology and microstructure of wings and external genitalia in Protohymenidae and Scytohymenidae. Moreover, we report the earliest evidence of Protohymenidae based on newly described species from the Mazon Creek Lagerstätte rooting the family age into Carboniferous. To conclude, our comparative analysis of Protohymenidae and Scytohymenidae uncovered structural details supporting the close relationship of Megasecoptera to Palaeodictyoptera and also uncovering number of homologies to Odonatoptera and Panephemeroptera.

Results

Superorder Palaeodictyopterida Grimaldi & Engel, 2005

Order Megasecoptera Brongniart, 1885

Family Protohymenidae Tillyard, 1924

Age range. Carboniferous (Middle Pennsylvanian, Moscovian) to Permian (Guadalupian, Roadian).

Emended diagnosis. Wings homonomous, petiolate, hindwing shorter. Arrangement of longitudinal veins of forewing and hindwing identical, CA+CP+ScA+ScP fused, running together towards wing apex, forming two rows of prominent spines on CA and ScA; CP and ScP running under CA and ScA; RA follows CA+CP+ScA+ScP, diverging in distal third of wing, than reaching wing margin, forming elongate (lanceolate) basally opened or closed apical cell; RP diverges from RA about midwing, ending with two branches; stem of M basally attached with CuA running close to RP in proximal part, than diverging in acute angle from radius towards the posterior wing margin, division of stem of M and CuA in basal third of wing length; single MA directed towards short anastomosis with RP, then ending in posterior margin, single MP ends in hind margin; CuA and CuP diverge very close to the wing base, CuA running basally attached to stem of M for a short distance than continue towards hind margin, single or terminally twigged CuP ends on posterior wing margin; single A1 present, cross-veins few in number 10–21, widely spaced.

Type genus. *Protohymen* Tillyard, 1924

Included genera. *Arroyohymen* Prokop & Kukalová-Peck, 2017; *Carbohymen* **gen. n.**, *Ivahymen* Martynov, 1932; *Permohymen* Tillyard, 1924; *Protohymen* Tillyard, 1924.

Remarks. We follow the systematics proposed by Carpenter [3] who considered the genus *Pseudohymen* Martynov, 1932 as junior synonym of *Protohymen* Tillyard, 1924 (*Pseudohymenopsis* Zallessky, 1956 was synonymised with *Pseudohymen* by Novokshonov [17]).

We agree with the synonymy of *Pseudohymen* with *Protohymen* for the following reasons: Until the published description of *Protohymen readi* Carpenter, 1933 (collected in 1932 at Elmo [18]) all species of *Protohymen* (from deposits in the U.S.A.) possessed only one crossvein between RA and RP1. On the other hand, all *Pseudohymen* species from Russian deposits had two crossveins on that place. But *Protohymen readi* (and also *Protohymen anomalus* Zimmerman, 1962 and *Protohymen pictus* Zimmerman, 1962, [19]) have also two crossveins on the same position. In addition, herein described *Protohymen novokshonovi* sp. n. from Tshekarda possesses also one crossvein, as the majority of *Protohymen* species from U.S.A. As the result, all species of *Protohymen* and *Pseudohymen* varies along size and shape of the wing only in arrangement and number of crossveins, which we consider as not sufficient character for separation of these two genera.

The structure of CuA is apomorphy for Protohymenidae among Megasecoptera. The CuA in related family Scytohymenidae is attached to free part of stem M for a very short distance, but the same course of CuA can be also seen in other families as Corydaloididae. The above mentioned character as seen in Scytohymenidae is probably ancestral state to the markedly longer connection of CuA to the stem of M as present in Protohymenidae. The course of CuA is also rather similar in some Diaphanopterodea (e.g. *Permodiapha carpenteri* Kukalová- Peck, 1974) where CuA runs for a short distance with stem of M (see Fig. 10 in [20]). On the other hand, this character is rather variable among Diaphanopterodea as there are differences in course of vein MA too ([21]; see Fig. 9 in [22]). So we consider similar arrangement of CuA at the wing base in Diaphanopterodea and Protohymenidae as a result of convergence.

Sunohymen Hong, 1985 [15] should be excluded from family Protohymenidae mainly because of the following differences in wing venation: *Sunohymen* lack anastomosis of MA with RP, veins are connected by a short crossvein, as in Bardohymenidae Zallesky, 1937 and other megasecopteran families (e.g. Aspidohymenidae and Foririidae). Another clear difference is two branched MP that is never present in Protohymenidae. Arrangement of apical part of RA is different too as it has no apical cell. According to diagnosis of Bardohymenidae given by Brauckmann *et al.* [23], the vein RP ends with 2–5 terminal branches and number of terminal branches of MP is not defined. Therefore, we suggest to transfer *Sunohymen* among Bardohymenidae.

Genus *Carbohymen* **gen. n.**

Etymology. Named after Carboniferous period and -*hymen* for membrane in Latin, masculine in gender.

Diagnosis. By monotypy, see that of type species.

Type species. *Carbohymen testai* **gen. et sp. n.**

Carbohymen testai **gen. et sp. n.**

Fig 1.

Etymology. Specific epithet honours Thomas V. Testa, collector of the holotype.

Diagnosis. Stem of M+CuA slightly remote from division of RA+RP, crossvein rp-m distinct; distal part of M+CuA and RA+RP form an acute angle about 50° in forewing; anal vein short.

Type material. Holotype TVT 1455 (originally in Thomas V. Testa coll., currently deposited in FMNH), positive (**B**) and negative imprint (**A**) of nearly complete specimen (female) preserved in siderite nodule.

Type locality. Mazon Creek, Peabody Coal Pit 11, Grundy County, Illinois, U.S.A.

Type strata. Francis Creek Shale Member, Carbondale Formation, Will-Kankakee-Grundy Co., Illinois, U.S.A.; Moscovian (315.2–307.0 Ma) ~ Westphalian C–D, Middle Pennsylvanian, Carboniferous [24].

Description. *Head*: Hypognathous with a short rostrum like mouthparts, detail structures poorly preserved (part B). *Thorax*: Prothorax reduced with small pointed winglets (A). Meso- and metathorax equal, wing articulation partly discernible. *Wings*: Homonomous, apical parts of all wings unknown, forewing (left, A): costal margin straight, CA+CP basally slightly remote from other veins, forming subtle hump; ScP runs along RA+RP, RP separates from RA at about half of wing length (6.5 mm from wing base), than directed towards hind margin of wing; stem of M basally runs near ScP+RA+RP, than diverge, but still remains connected to RA+RP by very short crossvein (rp-m, 0.2 mm), stem of M than split up, simple MA reaches RP (0.4 mm from division of RA) and anastomoses for 0.8 mm with RP, than direct to posterior margin; stem of Cu very short, simple CuA divide from CuP 0.6 mm from wing base, than runs with M, after 0.8 mm from diverging point of ScP+RA+RP, M separates from CuA, which runs toward posterior wing margin, simple CuP is parallel with CuA; single anal vein A1 short; crossveins arrangement: one between MP and CuA and between CuP and A1, two between CuA and CuP, one between A1 and posterior wing margin. Hindwing (left, A): venation pattern same as for forewing, A1 ending 4.3 mm from base, crossveins barely visible. *Abdomen:* Cylindrical with 10 abdominal segments, short ovipositor with valvulae V1 discernable (part B) emerging between segments VIII and IX, cerci with stout basal segments (part A).

Dimensions. Body length 20.3 mm (from the base of mouthparts to the end of 10th segment), estimated forewing length 15.4 mm (9.5 mm preserved) and maximum width 3.9 mm, hindwing preserved length 10.2 mm and maximum width 3.5 mm, thorax 6.4 mm, abdomen 12.5 mm.

Discussion. *Carbohymen* gen. n. clearly belongs to the family Protohymenidae because of shared characters in wing venation: (1) anterior margin of wing is formed by (CA+CP)+(ScA+ScP)+(RA+RP) those are connected into stiffened unit; (2) RP and MA anastomosed for short distance near mid-wing; (3) stem of M is basally attached to CuA; (4) veins MA, MP, CuA and A1 are simple.

General body morphology and dimensions correspond well with members of Protohymenidae, e. g. specifically short mouthparts, thoracic and abdominal morphology, relatively short ovipositor and basally enlarged cerci. We are not sure about the triangular structure that resembles prothoracic winglet (see Fig. 1A), but it certainly lacks traces of wing venation and it is discernable only on one side of prothorax. For that reason, we rather exclude this character from the diagnosis. Such prothoracic winglets are unknown among members of Megasecoptera so far, but these articulated structures occur in closely related group Palaeodictyoptera and also in Carboniferous stem group of dragonflies Geroptera [25]. Therefore, we can consider this character as plesiomorphy [26].

Genus *Carbohymen* gen. n. differs from *Arroyohymen, Ivahymen* and *Protohymen* by conspicuously shorter single anal vein which is character shared by *Permohymen*. Also all three genera with long anal vein have the value of angle between distal part of M+CuA and RA+RP under 30°, while *Carbohymen* gen. n. and *Permohymen* have this angle about 50°, which also corresponds to the less prominent petiolization in the latter two genera. *Carbohymen* gen. n. differs from *Permohymen* by hindwings nearly broad as forewings and wider space between stem of M and RA+RP, hence the crossvein rp-m is distinctly longer in *Carbohymen* gen. n. (Fig. 1, B,E).

Genus *Protohymen* Tillyard, 1924

Type species. *Protohymen permianus* Tillyard, 1924

Protohymen novokshonovi **sp. n.**

Figs 2,3.

Etymology. In honour and memory of Dr Viktor G. Novokshonov, palaeoentomologist and also collector of holotype.

Diagnosis. High number of crossveins: in total css 19 (forewing) and 21 (hindwing), three css between MP and CuA and four between CuA and CuP.

Type material. Holotype PIN 4987/115, positive (A) and negative (B) imprint of nearly complete specimen.

Type locality. Tshekarda lokality, Sylva River, Suksun District, Perm Region, Russian Federation [27].

Type strata. Koshelevka Formation; Kungurian (283.5–272.95 Ma), Cisuralian, Permian.

Description. *Head:* Compact head (length of head from epistomal ridge 1.5 mm. width 2.2 mm) with relatively large eliptical eyes (length 1.0 mm, width 0.6 mm); antennae with robust scapus and pedicel, flagellum with elongated flagellomeres, tentorium and base of mandibular stylets visible, epistomal suture discernable, located slightly before eyes (see Fig 2, E-G), distal section of mouthparts not preserved. *Thorax:* Prothorax reduced, narrower and shorter than meso- or metathorax, anterior edges of prothorax slightly pointed forward, prothoracic legs short attached anteriorly to prothorax, femur slightly shorter than tibia, meso- and metathorax similar in size. *Wings:* Homonomous, petiolate, forewings slightly longer. Wing venation (measurements of right forewing on part A): costal margin straight, CA+CP basally remote from ScA+ScP+RA+RP then gradually approach it, forming long triangular area; ScP originates close to RA, then runs along costal margin to wing apex; RA runs with CA+CP+ScA+ScP, then sharply diverges 3.9 mm from wing apex, running towards apex, forming closed apical cell (length 4.6 mm), RP runs basally alongside of RA, till diverging point 10.4 mm from wing base, free RP than curves and after 4.5 mm forks into two terminal branches RP1 and RP2; stem of M runs with CuA near ScP+RA+RP for 3.0 mm, than diverges from RA+RP (forming acute angle of 27° between RP and free stem-M), short crossvein rp-m near that point, M division 8.8 mm from wing base, single MA directed towards free RP, forming anastomosis with RP for 0.3 mm, than ends in posterior wing margin, single MP runs along MA reaching hind margin; stem of Cu short, after 2.7 mm CuP diverges, single CuA then runs with stem-M for 1.9 mm and continues to hind margin, single CuP follow CuA to hind margin; single anal vein 1A considerably long, ending in posterior wing margin 11.3 mm from wing base. Crossveins distribution: one between RA and RP1, one between RP1 and RP2, two (fw) or three (hw) between RP2 and MA, three between MA and MP, three between MP and CuA, four between CuA and CuP, three between CuP and A1, two (fw) or three (hw) between A1 and hind margin, total number of css 19 forewing (right on A), 21 hindwing (left on B); special crossvein rp-m is not counted in the sum. *Abdomen:* Segments I–VIII cylindrical, broader than long (length of the first segment 1.2 mm, width 2.8 mm).

Dimensions. Estimated total body length 21.5 mm, wingspan 50.5 mm, forewing length 23.2 mm, maximum width 5.0 mm, hindwing length 21.0 mm, maximum width 5.0 mm, preserved length of incomplete abdomen (I–VIII) 8.8 mm.

Discussion with emphasis on Protohymenidae morphology

Head. Tentorium is sclerotised X shaped structure of cephalic endoskeleton. It consists of body of the tentorium (or corpotentorium) with anterior and posterior arms which are hollow invaginations of head wall. Usually there are also dorsal arms present, which arise from the anterior arms [28].

On the holotype of *P. novokshonovi* sp. n., only anterior and posterior tentorial arms can be observed (Fig. 2E-G). Dorsal tentorial arms cannot be seen, probably due to their overlap with anterior arms or alternatively we should admit that dorsal arms are not developed. There is no discernable connection between left and right parts of tentorium, thus we cannot state if corpotentorium is developed or not. Distal parts of tentorial arms are often weakly sclerotised, thus it can be reason why these are not preserved in *P. novokshonovi.* The similar shape of anterior and posterior arms can be found for instance on recent mayfly nymph of *Eatonica schoutedeni* (Navás, 1911) (see Fig. 1 in [29]).

The position of posterior tentorial arms on *P. novokshonovi* suggests hypognathous position of mouthparts, because the posterior tentorial pits from which tentorial arms originate are usually located near occipital foramen. The thick longitudinal structure interpreted as upper tentorial arms was indicated in the revision of a diaphanopterodean *Permuralia* *maculata* (see Fig. 1 in [30]). However, the details as endoskelet in this species is not well preserved to allow closer comparison with *P. novokshonovi*.

Mouthparts. Herein described mouthpart of *P. novokshonovi* sp. n. belongs among the most interesting body structures and at the same time the best preserved in Megasecoptera. Not only because of the preserved tentorium, but the mouthpart bears particularly well discernable proximal part of mandibular stylets. These stylets are basally equipped with narrow parts curved outward, forming small hooks. The controlled abductor and adductor muscles were probably attached close to their bases. Similar shape of proximal part of mandibulae can be observed on recent Ephemeroptera like *Ameletopsis persitus* (Eaton, 1899) where its function is to articulate as posterior condyle with the cranium [31].

Mouthpart morphology has been recently described on few megasecopterans as *Brodioptera sinensis* and *Sinopalaeopteryx olivieri* [8, 9, 32]. Homologous structures of the mouthpart among Palaeodictyopterida can be also compared to *Permuralia maculata* (Kukalová-Peck & Sinichenkova, 1992) (Diaphanopterodea), where mandibulae are described as flat triangular structures pointing apically with sclerotised margins and small denticles along their inner margins [30]. Similar shape of madibulae seems to be present also in *Homoioptera vorhallensis* Brauckmann a Koch, 1982 (Palaeodictyoptera) (Fig. 137 in [33]). *Eugereon boeckingi* Dohrn, 1866 (Palaeodictyoptera) has a well preserved and long rostrum, but the basal parts of mandibular stylets are not of triangular shape albeit they are covered under sclerotised clypeus and frons [34]. In comparison with given examples, the basal parts of mandibular stylets are narrower and slender in *P. novokshonovi* sp. n., probably representing the type of mouthparts which was adapted for feeding on softer parts of plants like Cordaite seeds [35, 36].

Broader comparison of mouthpart morphology among representatives of all Palaeodictyopterida shows spectacular disparity in term of rostrum length and shape of mandibulary stylets probably reflecting adaptations for piercing and sucking on various Palaeozoic plant taxa.

Wings: Plasticity of venation characters among Protohymenidae

While the course and branching of longitudinal veins in Protohymenidae is rather conservative, the number of crossveins is often variable. Rare abnormalities concerning longitudinal veins appear in the material from Oklahoma, for instance *Protohymen* sp. (MCZ 4682) has deformed apical cell as RA apically anastomoses for a short distance with RP1. In paratype of *Ivahymen constrictus* PIN 3353/7 and also in *Protohymen bifurcatus* Zimmerman, 1962, RA is terminally twigged. The variability concerning anal vein was observed in single species of *Protohymen carpenteri* (specimen No. PIN 1700/3221) – anal vein is terminally twigged with crossvein in between these two branches.

Wings of *P. novokshonovi* and also holotype of *Permohymen schucherti* shows some individual variability in number of crossveins between forewing and hindwing. Previously noted intraspecific variability in the number of crossveins of some Protohymenidae was observed, for example the variation of number of crossveins between A1 and posterior wing margin in *P. permianus* with single or two css (p. 353 in [12]). Three crossveins between RA and RP1 were noticed on specimen PIN 1700/3224. The holotype of *Protohymen anomalus* Zimmerman, 1962 with one extra crossvein rp-m is also likely only abnormal specimen, as indicated by the authors [19].

Genera of Protohymenidae can be divided into two groups. The first group contains *Permohymen* and *Carbohymen* gen. nov., both can be characterized by broader wing base, short anal vein and proximally opened apical cell (putatively present also in *Carbohymen*, despite the wing apices of the holotype are not preserved). On the other hand, the second group comprising genera as *Arroyohymen, Protohymen* and *Ivahymen* can be distinguished by a narrow conspicuously petiolate wing base and closed apical cell. The basal third of the wings of the first group are slightly shortened, thus longitudinal veins diverge from costal margin in form of wider angle, specifically angle between basal part of M+CuA and RA+RP of value about 50°. Remaining three genera have value of the same angle less than 30°.

Newly described *Carbohymen* gen. n. differs from *Permohymen* mainly by wider space between stem of M and RA+RP with distinctly developed rp-m. Genus *Arroyohymen* can be separated also by dark pterostigma in basal part of apical cell and short middle part of M (see [37]). Genus *Ivahymen* differs from *Protohymen* by distally forked CuP. The most species rich genus *Protohymen* (with 18 species according to this study) comprise species with closed apical cell on wings and without pterostigma (see Fig. 4 for details of type species *Protohymen permianus*).

Regular veinal corrugation is typical for all members of Palaeoptera, but it is not always clearly visible. It is well discernable in several species of Protohymenidae, for example in the holotype of *Protohymen venustus* Carpenter, 1947.

Pterostigma and apical cell in Protohymenidae

According to the definition given by Norberg [38], a pterostigma is pigmented spot close to the leading edge in the apex of insect wing. This area is bigger in mass and it prevents the wing against self vibrations arrising at certain speed. In small insects, the pterostigma makes slow and hovering flight more efficient by facilitate better pitch angle control. The slow hovering flight is also assumed for members of Protohymenidae, as for other Megasecoptera with petiolate wings [39].

The wing venation of Protohymenidae was firstly described by Tillyard [10]. Tillyard's definition of pterostigmal area includes also jointed veins of costal margin. Carpenter [12] revised the wing venation of Protohymenidae. In his subsequent work [14], he compared his interpretation with the one of Martynov [13] and proposed a new conception of the wing apex. Carpenter [14] suggested that RA has a short anterior branch that merges with anterior wing margin, but alternatively the same structure can be considered as a crossvein.

We follow the interpretation of Carpenter with minor modifications. In our opinion the genus *Permohymen* has dark pterostigmal area that can be observed between apical sections of CA+ScA and RA (see Fig. 5A–C). In *Protohymen* and *Ivahymen* the pterostigma is completely reduced and replaced by a short reinforced anterior branch of RA merging with the leading edge (see Fig. 2D, 4D). The apical cell is located between the posterior branch of RA and composite leading edge of anterior wing margin. Zalessky [40] proposed a theory on the development of veinal arrangement in apical part of wing for four megasecopteran families (Aspidohymenidae, Aspidothoracidae, Bardohymenidae and Protohymenidae).

Legs. Among species of Megasecoptera only prothoracic legs are usually preserved as known in Bardohymenidae, Mischopteridae, Protohymenidae, Scytohymenidae and Vorkutiidae [41]. Forelegs of Protohymenidae are relatively short and shifted anteriorly like in Mischopteridae and others probably helping insect during feeding and with manipulation of elongated mouthparts [1, 8, 9]. Therefore, we can consider the correlation between the length of mouthparts and forelegs.

Prothoracic legs with tarsi consisted of 5 segments is well preserved on *Protohymen permianus* MCZ 3060a (see Fig. 4E–G). This number of tarsomeres is presumed as plesiomorphic state for Pterygota as it occurs in its sister group Zygentoma with basal Lepidotrichidae [42].

Genitalia. The ovipositor in Protohymenidae is preserved in lateral aspect only in one specimen of *Permohymen schucherti* (Fig. 5E,F; specimen MCZ 3815). The abdominal segment IX is clearly enlarged as it contains large muscles that control the ovipositor, albeit no internal structures can be discerned. Valvulae V1 and V2 surrounds gonoporus and both are preserved in basal parts with their apices incomplete. Sheathing valvulae V3 cannot be observed. The segment X is shorter bearing a pair of basally stout cerci (Fig. 5,F). Basally broad and relatively short ovipositor is common feature of Protohymenidae, Scytohymenidae and also Vorkutiidae among megasecopterans as noted by Novokshonov [41].On the other hand, an ovipositor of *Brodioptera sinensis* shows long and straight valvulae reaching the apex of prolonged terminal abdominal segment [8, 9]. Another known ovipositor of megasecopteron *Foriria maculata* Meunier, 1908 is relatively long and slightly curved [43]. Ovipositor of *Asthenohynem dunbari* Tillyard, 1924 (Diaphanopterodea) formerly described as protohymenopteron *Doter minor* Sellards, 1907, is depicted by Carpenter [44]. The third sheathing valvulae (V3) of the latter species clearly exceed the length of abdomen.

The male external genitalia in Protohymenidae are preserved in several specimens like in holotype and paratype of *Protohymen carpenteri*, but the best preserved can be observed in *Permohymen schucherti* (Fig. 5G,H; specimen MCZ 3819a). The latter specimen has been already studied and briefly described by Carpenter [45], who pointed the resemblance with genitalia of Ephemeroptera. Recently, the male genitalia of *P. schucherti* were compared to *Brodioptera sinensis* (Megasecoptera: Brodiopteridae) [9]. The presence of curved forceps with tubercles or setae along the inner margin is common character of Ephemeroptera and Megasecoptera. The paired pennis as a typical feature of Ephemeroptera is considered as putative synapomorphy of Palaeoptera (see [26]) and can be found also in *P. schucherti.* The penial lobes are stout and short, with longitudinal sclerotised structures, which we interpret as seminal grooves (see Fig. 5G,H). These grooves are situated on the apical part of penial lobes of recent Ephemeroptera [46, 47].

Family **Scytohymenidae** Martynov, 1937

Diagnosis. As proposed by Shcherbakov *et al.* [36] with some newly included characters: ScP running along RA, RA thickened running close to costal margin; veins RA, MA, MP, CuA and CuP simple; RP with two terminal branches; presence of short oblique crossvein rp-m behind the divergence of M from RA+RP; MA anastomosed for short distance with RP, CuA anastomosed with MP; anal vein pectinate ending with at least two posterior branches; few crossveins faintly preserved.

Type genus. *Scytohymen* Martynov, 1937

Included genera. *Oceanoptera* Shcherbakov, 2009, *Scytohymen* Martynov, 1937, *Tshekardohymen* Rohdendorf, 1940.

Remarks. Martynov [13] included family Scytohymenidae into the order Protohymenoptera based on branching pattern of veins RP, M and Cu together with Protohymenidae and Asthenohymenidae. The latter family was later transferred into order Diaphanopterodea due to ability of wing folding [21]. Members of family Scytohymenidae have different organization of vein RA and the course of CuA as the main diagnostic characters in contrast to Protohymenidae.

Scytohymen extremus Martynov, 1937

Fig 6.

Material. Supplementary specimen No. PIN 4987/113 (originally PU 1/143). Nearly completely preserved specimen (positive B and negative imprint A) with head, thorax, right forewing and right hindwing, abdomen with endophytic ovipositor.

Locality. Tshekarda lokality, Sylva River, Suksun District, Perm Region, Russian Federation [27].

Strata. Koshelevka Formation, Kungurian (283.5–272.95 Ma), Cisuralian, Permian.

Description.

Head: Hypognathous, conspicuously bulging compound eyes with discernible pattern of hexagonal shaped ommatidia (approx. 40 × 25), filliform antennae very long with broad scapus + pedicel. *Thorax:* Robust, prothorax reduced with distally incomplete leg positioned anteriorly, prothoracic leg with relatively short femur and slender tibia. *Forewing:* costal margin straight, ScP running basally with RA+RP ending (merging) into costal margin near wing apex, RA+RP running straight along costal margin, RA apically gradually deflects from C+ScP ending in wing apex, RP sharply diverges from RA 20.8 mm from wing base, RP shortly free for 1.6 mm behind the point of divergence with RA, than RP shortly coalesced with MA, after that it again markedly change direction and goes almost parallelly with RA for 5.2 mm, than split into two terminal branches; stem of M runs near RA+RP, after 6.8 mm from wing base it diverges distinctively from RP, but still it is connected by short crossvein r-m, it is shortly anastomosed with CuA, M then directed towards wing apex, split into anterior and posterior branches 14.9 mm from wing base, single MA runs towards free RP and anastomosed with it for 0.9 mm, than ends in hind margin, simple MP gradually proceeding towards posterior margin, stem of Cu basally runs near stem M for 2.3 mm, than diverges gradually and split up into anterior and posterior branch 4.5 mm from wing base, free single CuA for 2.7 mm further anastomosed with M for 1.1 mm, than it ends in posterior wing margin; CuP simple, distinctly shorter than CuA ends in posterior wing margin; single short A1 posteriorly pectinate ending with two terminal branches. *Abdomen:* With 10 visible segments, relatively short ovipositor emerging between abdominal segments VIII and IX.

Dimensions. Body length 28.1 mm, length of antennae (incomplete) 12.7 mm, right eye length 1154 μm (cca 40 ommatidia), width 687 μm (cca 25 ommatidia), diameter of single ommatidium cca 45 μm , forewing length 40.0 mm and maximum width 8.7 mm, hindwing length 37.2 mm and maximum width 9.0 mm, thorax length 8.5 mm, prothorax length 2.2 mm, pterothorax length 6.3 mm, abdomen length 17.2 mm.

Discussion.

Martynov [13] established the family Scytohymenidae based on holotype of monotypic genus *Scytohymen* (with type species *S. extremus* Martynov, 1937) which has not preserved the most basal part of wing and other body structures. Later, the genus *Tshekardohymen* was described by Rohdendorf [48] who also based the holotype on isolated wing. Shcherbakov *et al.* [36] given the revised diagnosis and established the third genus *Oceanoptera*, also on the basis of isolated wing. Here we present additional nearly complete specimen of *Scytohymen extremus* that contributes significantly to the knowledge on body morphology of this family previously unknown.

This newly described specimen was already noted by his collector Viktor G. Novokshonov as nearly complete specimen of *Tshekardohymen martynovi* Rohdendorf, 1940, however without indication of the collection number [41]. As pointed by Shcherbakov *et al.* [36] *Scytohymen* and *Tshekardohymen* both share similar pattern of veins to each other than outlined by Rohdendorf [48]. We consider the placement of the present specimen to *S. extremus* due to the corresponding pattern of branching main longitudinal veins and also wing dimensions of the holotype No. PIN 2/99. On the other hand, the wing of *Tshekardohymen* is slightly smaller (estimated wing length about 35 mm) and has darkly coloured wing membrane (Fig. 6G). Furthermore, there is slight difference in wing shape and number of crossveins between the holotype and supplementary specimen of *S. extremus*, but it can be attributed to intraspecific variability or possibly taphonomy reasons only. Crossveins can be distinguished very poorly in both specimens of *S. extremus* and also among described species of *Tshekardohymen* and *Oceanoptera*. In some species of Scytohymenidae (see Fig. 3 in [36]) there are visible striae or wrinkles on wing membrane supposedly due to taphonomy reasons as low number of crossveins could affect tension on the wing membrane as can be also seen on the holotype of *Issadohymen ponomarenkoi* Sinitshenkova & Aristov, 2013 (Megasecoptera: Moravohymenidae [49]). The supplementary specimen also provided new data on veinal architecture of anal area that was previously unknown in Scytohymenidae.

The visible detail structure of bulging compound eyes of half-elipsoid shape is documented for the first time in Megasecoptera and probably also among Palaeodictyopterida. Our examination confirms the array of relatively large hexagonal ommatidia closely spaced with approximate diameter of each facet about 45 μm preserved on dorsal part of compound eyes (Fig. 6C,D). On the basis of preserved parts we suspect that all ommatidia were similar in size and compound eyes were probably not divided like for instance in some anisopteran dragonflies or males of mayflies with dorsal ommatidia having distinctly larger dorsal facets [50, 51]. Therefore, we could roughly estimated the density counting about 800 facets per millimeter squared and the total number of facets on each eye spanning between one and two thousands. The larger ommatidia in Anisoptera are present on species those are active during twilight [50]. Therefore, we cannot exclude the scenario for these megasecopterans of activity in low light conditions or possibly at twillight.

Conclusions

Our study allowed a reconstruction of close up morphology of various body parts on extinct megasecopteran families Protohymenidae and Scytohymenidae. To summarize our findings, we show hypognathous head with endoskeleton as tentorium, mouthpart in form of a short rostrum uncovering proximal part of mandibulary stylets controlled by muscles, surface of compound eyes consisted of hexagonal pattern of large facets, structure and microstructure on wings, and finally male and female external genitalia. For the first time we report the earliest species of Protohymenidae from the late Carboniferous siderite nodule of Mazon Creek in Illinois, USA.

Our study also provides insights into functional morphology of mouthpart stylets as a unique synapomorphy for the Late Paleozoic Palaeodictyopterida, form of tentorium, vision provided by large hexagonal ommatidia possibly reflecting activity during twilight for these insects, structural and microstructural details on wings like composite anterior wing margin, development of apical cell and external genitalia like male curved claspers to hold female during copulation and paired penial lobes with seminal grooves. Based on the obtained results and comparison of homologous structures known on extant relatives of Ephemeroptera and Odonata we assumed the close relationship for Panephemeroptera, Odonatoptera and Palaeodictyopterida and thus support for monophyly Palaeoptera.

Methods

Line drawings and photographs

The venation patterns were drawn directly through a stereomicroscope Leica MZ 12.5 with the aid of a camera lucida. Habitus reconstruction of *Protohymen novokshonovi* was based on line drawings colored by acrylic paints subsequently. Photographs were taken with attached digital camera Canon D550 with lenses EF 50 mm and MP-E 65 mm. Original photographs were processed using the image-editing software Adobe Photoshop CS4, and for some images were processed by the focus-stacking software Helicon Focus Pro. Scanning electron micrographs were taken by an environmental electron microscope Hitachi S-3700N at the National Museum in Prague.

Wing venation terminology

We follow the interpretation of composition leading edge of wing as proposed for Odonata by Riek & Kukalová-Peck [52] and Bechly [53]. We consider the veins between single anal vein and posterior wing margin as crossveins according to Carpenter [12].The terminology used for the description of the microstructures follows D'Andrea, M. & Carfi [54, 55].

Anatomical abbreviations

The following symbols are used for the wing veins (symbols in capitals denote the longitudinal veins): CA / CP – costal anterior / posterior, ScA / ScP – subcostal anterior / posterior, RA / RP – radial anterior / posterior, MA / MP – medial anterior / posterior, CuA / CuP – cubital anterior / posterior, A1 – anal vein; brace rp-m - short crossveins.

The following symbols are used for other body structures: abdominal segments VII-X; ac - apical cell; at - anterior tentorial arms; ce - cerci; cl - clypeus; cw - claw; css - crossvein/ crossveins; er - epistomal ridge; ey - compound eyes; fc - forceps; fw - forewing; hw- hingwing; ms - mandibular stylets; ovi - ovipositor; pl - penial lobes; ps - pterostigma; pt - posterior tentorial arms; sg - seminal grooves; tarsal segments 1-5; V1/V2 - first and second pair of ovipositor cutting valvulae.

Fossil material

The specimens from the following institutional collections were examined: **FMNH**: Field Museum of Natural History (Paleontology) in Chicago, U.S.A.; **MCZ**: Museum of Comparative Zoology at Harvard University (Cambridge, MA); **PIN**: Paleontological Institute of Russian Academy of Sciences, Moscow, Russia; **PU**: Perm University, Perm, Russia; **YPM**: Yale Peabody Museum (New Haven, USA).

Significance of collection prefix in PIN: The material studied above was gathered by E.V. Permyakova in 1935–1936 (prefix 118) and in 1937 (prefix 168), during expeditions headed by A.G. Sharov from 1959 to 1961 (prefix 1700) and finally collected by V.G. Novokshonov from 1989 to 2000 (prefix 4987). All of these collections came from Tshekarda locality near Sylva River. Details on the locality can be found in Aristov [56] and Ponomaryova *et al.* [27]. List of available specimens of Protohymenidae for detailed study: PIN 1700: 4153 (holotype of *P. carpenteri*), 3226 (paratype of *P.carpenteri*), 407,412,422,440,445,448, 3231,479,480, PIN 4987: 115. Material of Scytohymenidae: PIN 4987/113 (= PU 1/143).

Remarks on studied material

The described paratype of *Protohymen permianus* (YPM 5002, as mentioned by Carpenter [12]) is not in fact the paratype, but a counterimprint of the holotype (YPM 5001). The examined specimen MCZ 29977 is a counterpart of the holotype *Protohymen venustus* (MCZ 4668). Therefore, we suggest indication of corresponding part and counterpart in the collections catalogues.

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Availability of data and materials

All data related to this study are presented in the paper.

Authors' contributions

M.P. conceived the initial idea and designed the project. Both authors wrote the paper and contributed to the interpretations and discussions. MP prepared line drawings and photographed the specimens. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

Consent for publication

Not applicable

Ethics approval

Not applicable

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Figure captions

Figure 1. *Carbohymen testai* gen. *et* sp. n., holotype No. TVT 1455, AB. **A,** photograph of habitus (part A, horizontally reversed); **B,** composite line drawing of part A and B; **C,** detail of basal segment of cerci, part A; **D,** detail of ovipositor, preserved ventrally, part B; **E**, detail of basal parts of wings with marked course of CuA. [ce - cerci, V1 - first pair of ovipositor valvulae]

Figure 2. *Protohymen novokshonovi* sp. n., holotype specimen PIN 4987/115; **A**, photograph of habitus (part A, obverse, positive); **B**, composite drawing of part A and B; **C**, detail of basal part of right forewing (A); **D**, SEM of apex of right forewing (A); **E,F** photograph of detail of head (A); **E,** SEM of head (A); **F**, reconstruction of head external and internal morphology. [ac - apical cell, at - anterior tentorial arm, cl - clypeus, er - epistomal ridge, ey - compound eye, ms - mandibular stylets, mu - muscle, pt - posterior tentorial arm, tn - tentorium]

Figure 3. *Protohymen novokshonovi* sp. n., habitus reconstruction, based mainly on holotype PIN 4987/115, length and morphology of distal section of mouthparts taken from specimen PIN 1700/445 and 1700/3231 of *P. carpenteri* and MCZ 3060a *Protohymen permianus*, morphology of maxillary palpae from 1700/422 of *P. carpenteri,* lenght and morphology of antennae and cerci from MCZ 3262a *Protohymen elongatus*.

Figure 4. *Protohymen permianus* Tillyard, 1924. **A-D**, holotype specimen YPM 5001; **A**, photograph; **B**, drawing; **C**, detail of apical part of wing with indication of D; **D**, detail of separation of vein RA and serrate CA+ScA; **E-F**, specimen MCZ 3060ab; **E**, photograph of part a; **F**, drawing. [1-5 - tarsal segments, ac - apical cell, cw - claws]

Figure 5. *Permohymen schucherti* Tillyard, 1924. **A-C**, holotype specimen YPM 5003a; **A**, photograph; **B**, drawing; **C**, SEM detail of apical part of wing, with dark sclerotized area between ScP and RA labeled as pterostigma ; **D**, specimen MCZ 3258a, wing base; **E,F** specimen MCZ 3815, female; **E,** detail of ovipositor; **F**, drawing; **G,H** specimen MCZ 3819a, male; **G**, detail of genitalia; **H**, drawing. [VII-X - abdominal segments, ac - apical cell, ce - cerci, fc - forceps, pl - penial lobes, ps - pterostigma, sg - seminal groove, V1/V2 - first and second pair of ovipositor valvulae]

Figure 6. Family Scytohymenidae **A-F**, *Scytohymen extremus* Martynov, 1937, specimen PIN 4987/113; **A**, photograph (part A, negative, horizontally flipped); **B**, composite drawing (part A+B); **C**, detail of head; **D**, detail of ommatidia; **E,** detail of basal segments of antennae; **F**, detail of basal part of forewing (part B, positive); **G**, *Tshekardohymen martynovi* Rohdendorf, 1940, photograph of holotype specimen PIN 212/25, flipped horizontally; **H-I**, *Scytohymen extremus* Martynov, 1937, holotype specimen PIN 2/99; **H**, photograph; **I**, drawing. [ovi - ovipositor, pc - pedicel, sc - scapus].

Figure 1

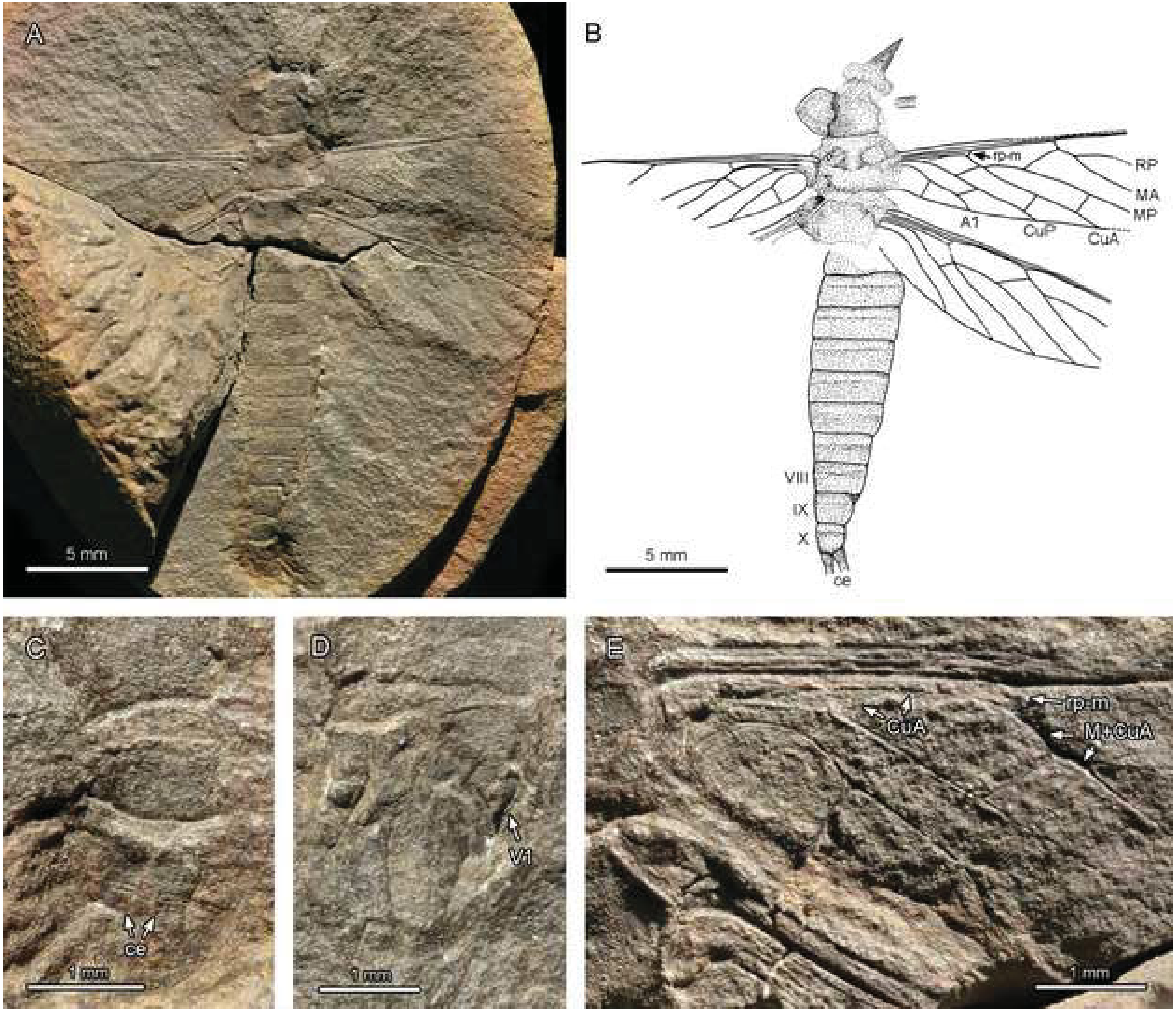
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Figure 2

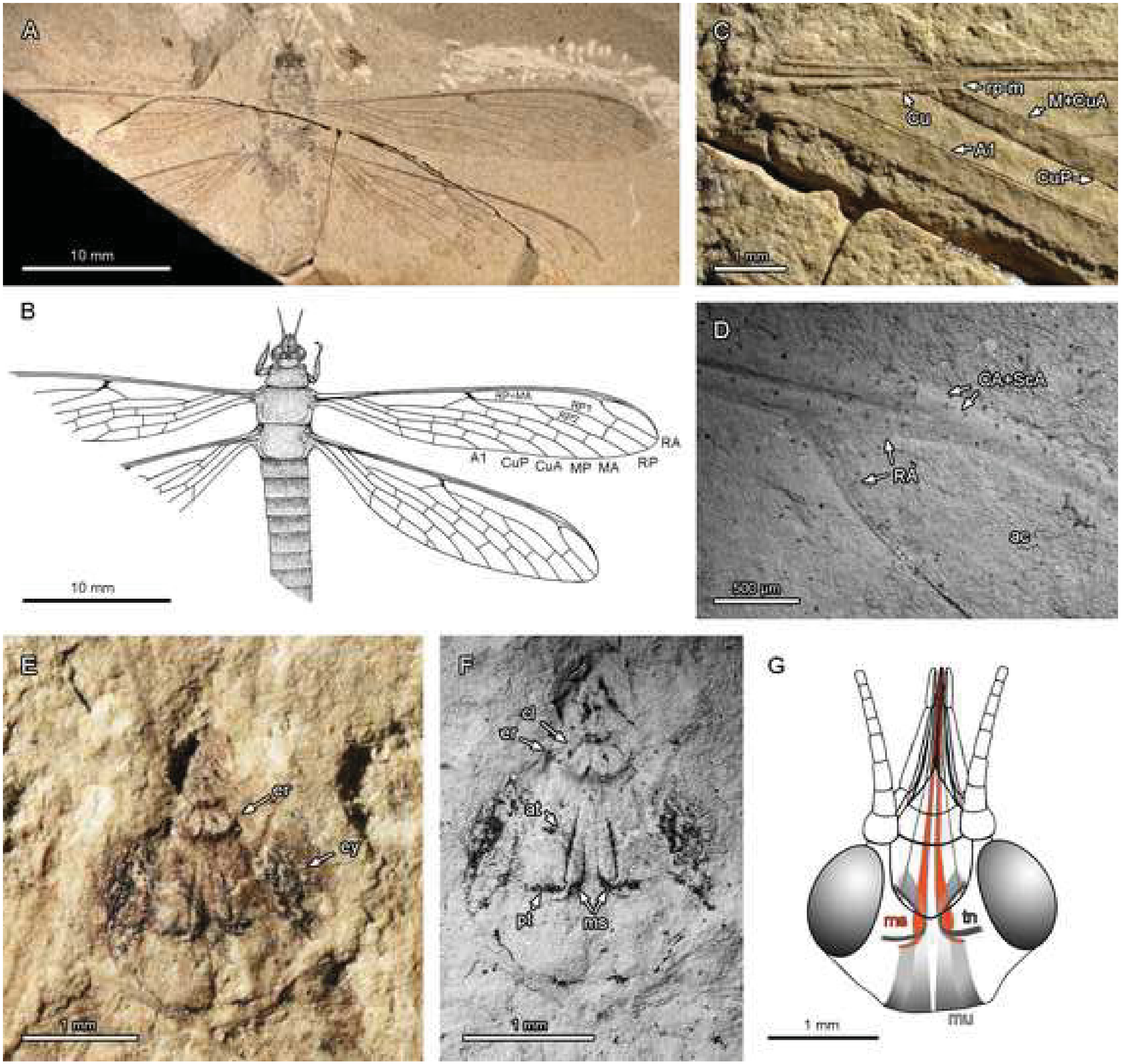
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Figure 3

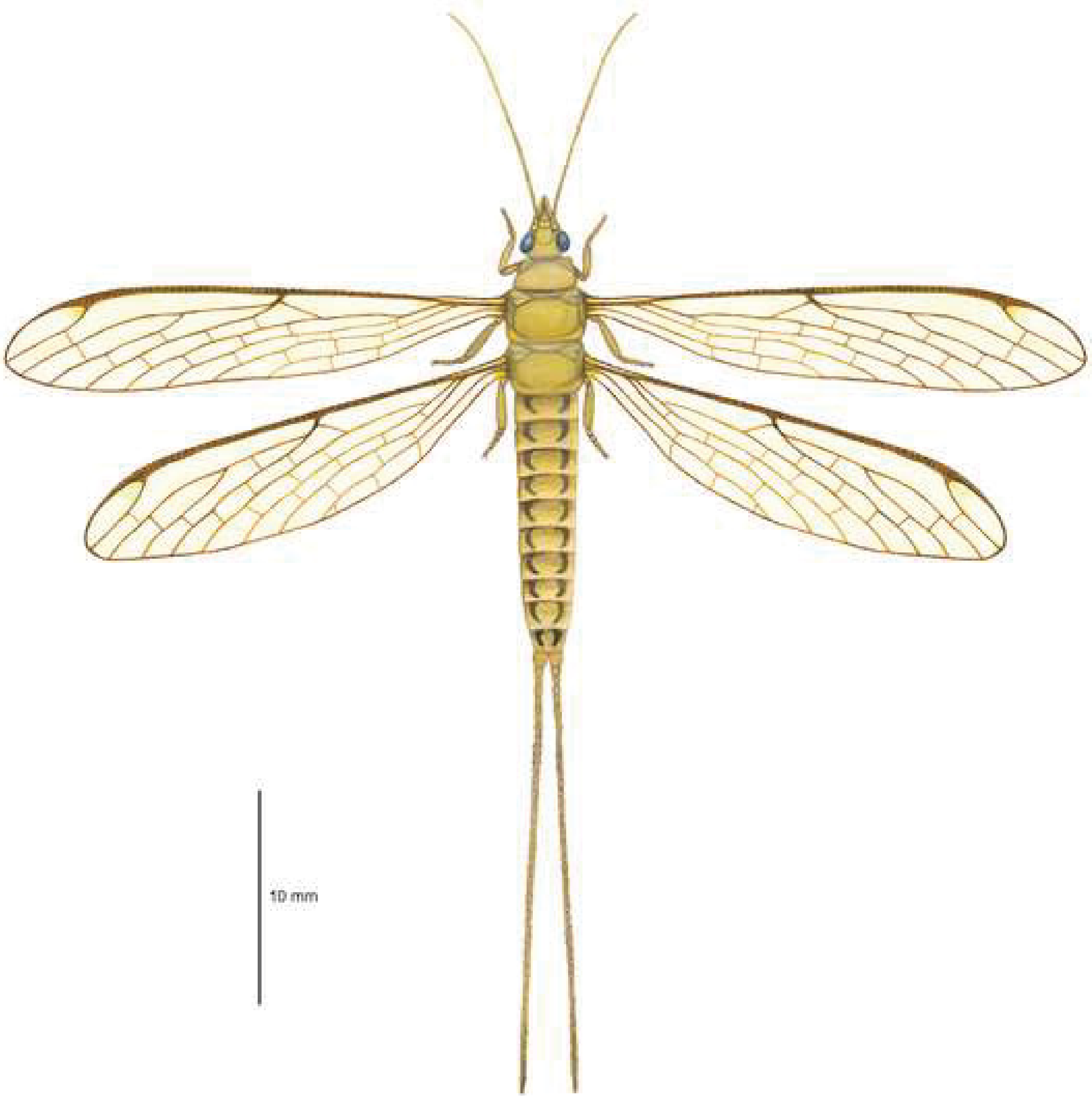

Figure 4

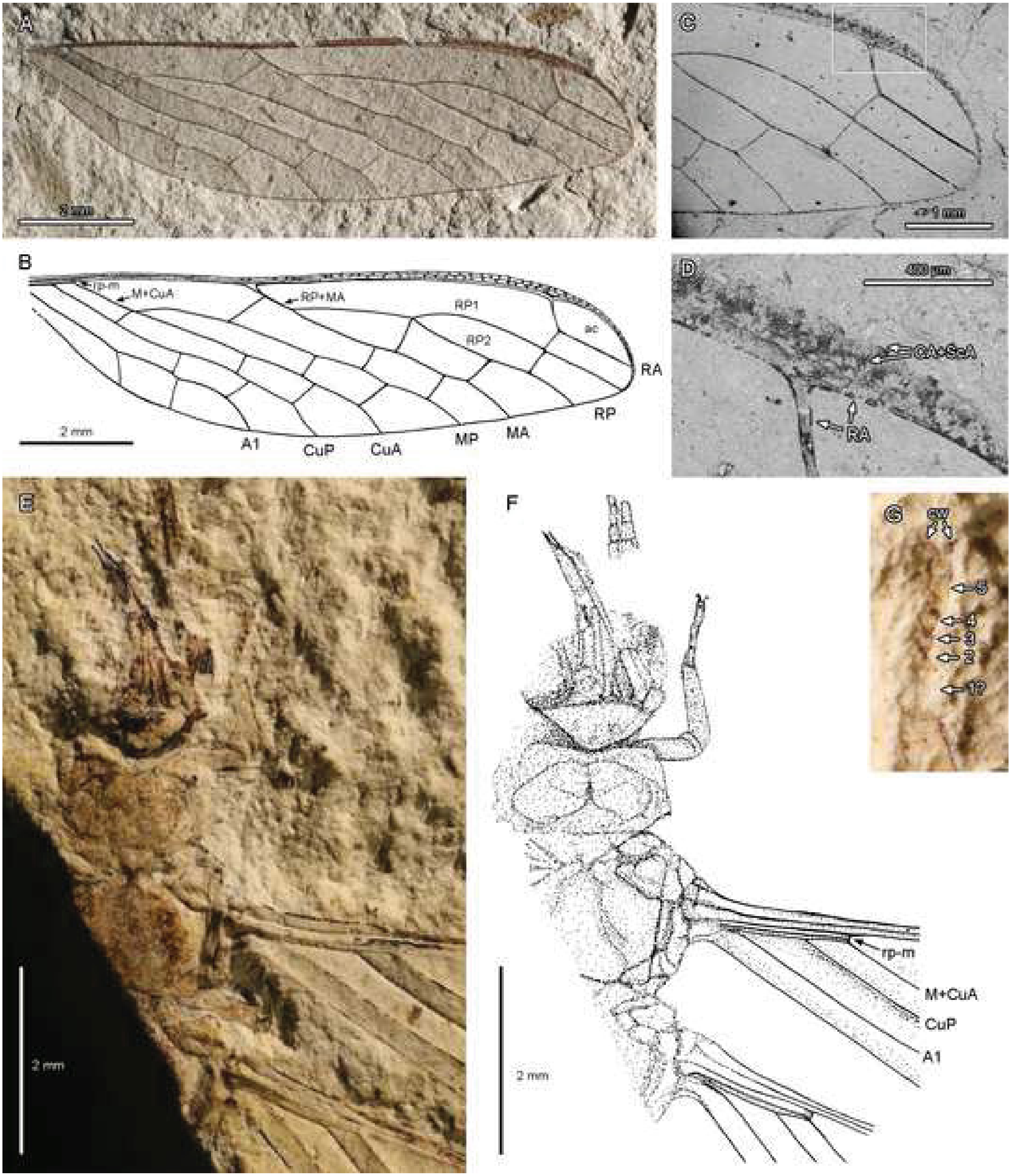
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Figure 5

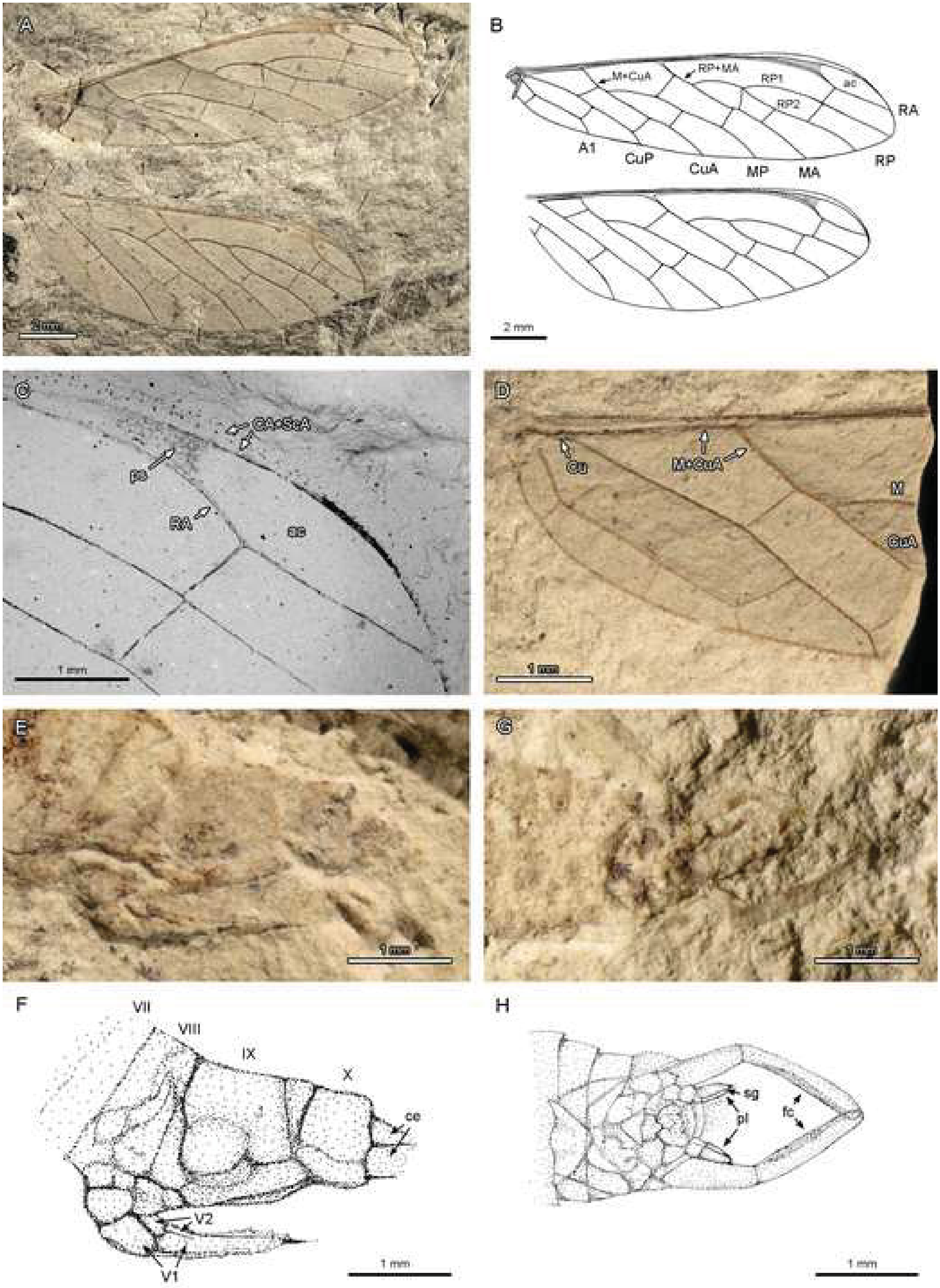
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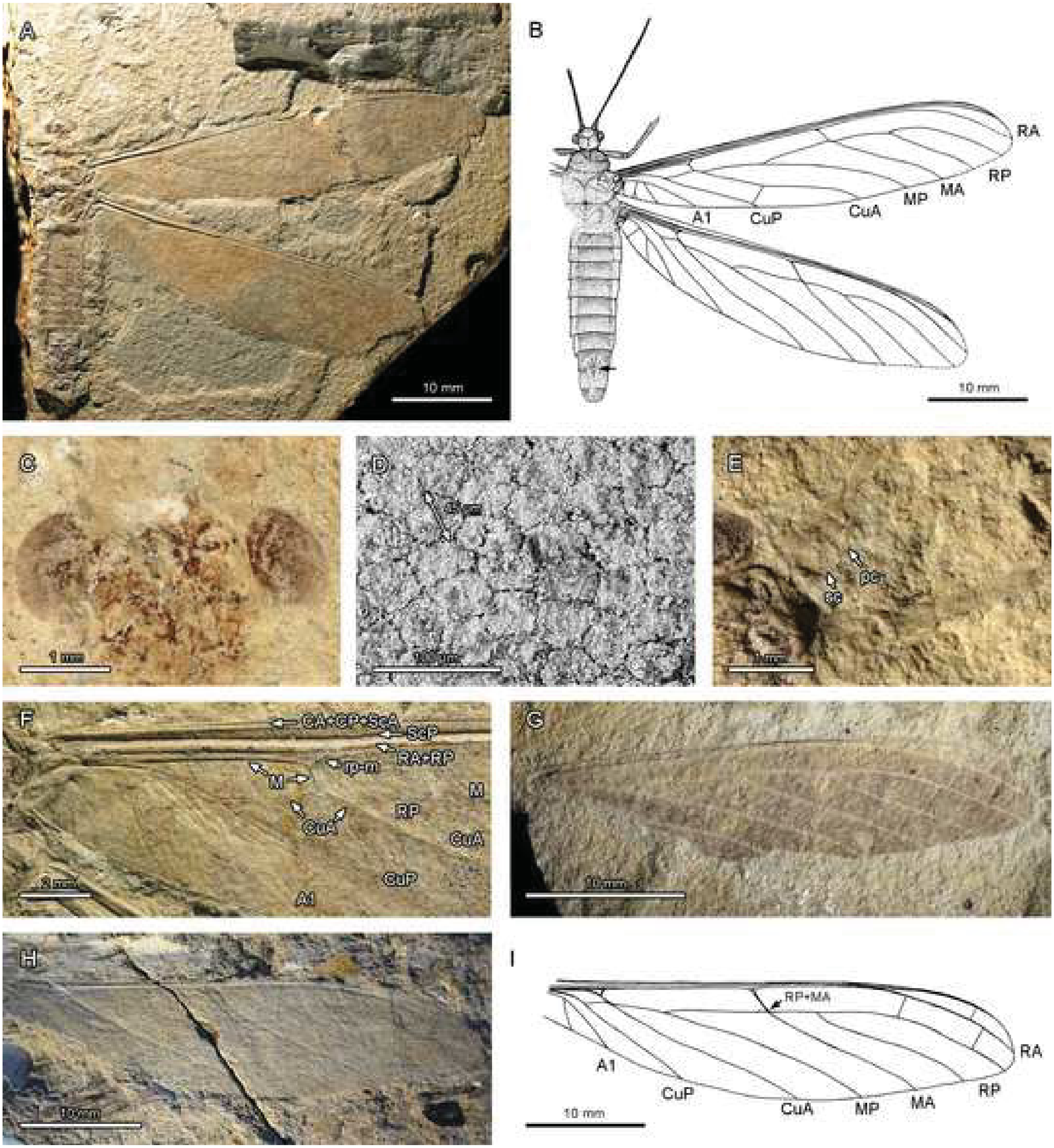
Figure 6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Článek 2

Pecharová, M., Ren, D. & Prokop, J. 2015. A new palaeodictyopteroid (Megasecoptera: Brodiopteridae) from the Early Pennsylvanian of northern China reveals unique morphological traits and intra-specific variability. *Alcheringa* 39: 236–249.

Podíl autorů: MP: studium materiálu, dokumentace, texty: popisy, diskuze, výsledky, obrazové tabule, fotografie, kresby; **DR**: zapůjčení materiálu, informace o lokalitě; **JP**: korespondenční autor, texty: úvodní kapitoly, shrnutí, část fotografií.

A new palaeodictyopteroid (Megasecoptera: Brodiopteridae) from the Early Pennsylvanian of northern China reveals unique morphological traits and intra-specific variability

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PECHAROVÁ, M., REN, D. & PROKOP, J., 29.01.2015. A new palaeodictyopteroid (Megasecoptera: Brodiopteridae) from the Early Pennsylvanian of northern China reveals unique morphological traits and intra-specific variability. *Alcheringa 39*, 236–249. ISSN 0311-5518

Evaluation of abundant insect fossils from the Early Pennsylvanian (Namurian) Tupo Formation of northern China (Xiaheyan locality, Ningxia Hui Autonomous Region) enabled description of a new megasecopteran, i.e., *Brodioptera sinensis* sp. nov. and emendation of Brodiopteridae. *Brodioptera sinensis* is defined principally on wing venation characters and darkly pigmented wing apices, but a few have hyaline wing membranes possibly reflecting freshly emerged imagoes or subimagoes without fully developed coloration. The large assemblage of 54 specimens enabled documentation of intra-specific variability in wing venation. In addition, the new species reveals the detailed morphology of the haustellate mouthparts with conspicuous elongated stylets, and external copulatory organs that, previously, were poorly documented or unknown. The male and female external genitalia are similar to members of Protohymenidae (Megasecoptera) and Asthenohymenidae (Diaphanopterodea). *Brodioptera sinensis* is related to *Brodioptera stricklani* and *Brodioptera cumberlandensis* both known from localities in Euramerica suggesting faunal exchange occurred during the Bashkirian. Moreover, based on our analysis of veinal characters and re-examination of the holotype of *Sylvohymen pintoi* Brauckmann known from Namurian of Hagen-Vorhalle (Germany), we propose transferring this taxon to Brodiopteridae as *Brodioptera pintoi* comb. nov. Despite the different palaeoclimatic conditions in Euroamerica and Gondwanaland, we assume that Brodiopteridae are closely related to Xenopteridae (type genus: *Xenoptera*) known solely from South America because these groups have similar venation patterns and were contemporaneous.

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Key words: Palaeodictyopteroida, Mischopterida, Brodioptera, new species, external copulatory organs, Upper Carboniferous, Bashkirian, Ningxia, Xiaheyan, China.

MEGASECOPTERA belongs to the late Palaeozoic Palaeodictyopteroida, broadly considered to be the sister group of Eupalaeoptera or Hydropalaeoptera (Ephemeroptera + Odonatoptera) within Palaeoptera. All members of Palaeodictyopteroida comprising Diaphanopterodea, Megasecoptera, Palaeodictyoptera and Permothemistida share the synapomorphy of uniquely modified haustellate mouthparts in the form of a rostrum with five prominent stylets, which led to their alternative name Rostropalaeoptera (Kukalová-Peck 1991, Wootton & Kukalová-Peck 2000, Bechly 2007). Palaeodictyopteroida reached astonishing diversity and disparity during the Pennsylvanian and Permian but did not survive the end-Permian mass extinction. The marked similarities in wing venation and body structures indicate a very close relationship between the orders Megasecoptera and Palaeodictyoptera (e.g., Carpenter 1962, Kukalová-Peck 1974, Sinitshenkova 1980). However, the ordinal status of Megasecoptera is currently in question owing to a lack of autapomorphies, and it seems to be a specialized lineage of Palaeodictyoptera with homonomous, basally narrow and commonly petiolate wings adapted to slow flight (Carpenter 1992, p. 46, Wootton & Kukalová-Peck 2000, Engel *et al*. 2013).

The systematics of Megasecoptera are unresolved, with some families, such as Eubleptidae, Ancopteridae, variably placed either in this group or in Palaeodictyoptera. Riek (1976) demonstrated, using *Psychroptilus burretae* from the Pennsylvanian of Tasmania, the difficulty with ordinal placement of this taxon, which has a mosaic of characters of both groups, and this resulted in his designation of a new megasecopteran suborder Neosecoptera. The same author proposed a key to the families of Eumegasecoptera and Protohymenoptera based on wing venation characters. Sinitshenkova (2002, pp. 107, 121) proposed a clade of ordinal rank (Mischopterida) comprising Megasecoptera as sister group of Archodonata based on some assumed synapomorphies. This order is subdivided into four suborders: Eubleptina, Mischopterina, Aspidothoracina and

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Permothemistina. Several families were also transferred to Dictyoneurida (=Palaeodictyoptera). Nevertheless, this system was not based on a phylogenetic analysis.

Carpenter (1963) established monotypic Brodiopteridae for *Brodioptera* Copeland, 1957 separating the family from Sphecopteridae and Corydaloididae, which were considered closely related groups. Two species, i.e., *B. cumberlandensis* Copeland, 1957 and *B. stricklani* Nelson & Tidwell, 1987, are presently assigned to this genus; both are known from the Bashkirian (Namurian B) of North America.

Carboniferous insects from Xiaheyan in Ningxia Autonomous region, China (the so called Qilianshan entomofauna), have been studied intensively for about a decade. Insects are preserved in terrestrial facies of the Tupo Formation dated to Namurian B/C (early Bashkirian; see Lu *et al*. 2002, Zhang *et al*. 2012). Owing to intensive fieldwork in the past few years and description of numerous new taxa, the locality has become an important source of data on rare Early Pennsylvanian insects (Namurian). Taxonomic studies have focused on members of the following insect groups: Palaeodictyoptera (e.g., Prokop & Ren 2007, Li *et al*. 2013b), Odonatoptera (Ren *et al*. 2008, Li *et al*. 2013a), stem-Grylloblatodea (Peng *et al*. 2005), stem-Dictyoptera (e.g., Wei *et al*. 2013), stem-Orthoptera (e.g., Liu *et al*. 2009, Béthoux *et al*. 2012a, b) and stem-Plecoptera (Béthoux *et al*. 2011). Some of the abundant species, such as *Sinonamuropteris ningxiaiensis* Peng *et al*. 2005 (Grylloblattodea) and *Longzhua loculata* Gu *et al*. 2011 (Archaeorthoptera), are represented by tens of specimens, allowing evaluation of the intra-specific variability (Cui *et al*. 2011, Gu *et al*. 2011).

Material and methods

All specimens are housed in the Key Laboratory of Insect Evolution and Environmental Changes at Capital Normal University (prefix CNU-) in Beijing (China). The material consists of 54 compressed fossil specimens in various states of preservation dominated by isolated wings or their fragments (34 specimens), but also more or less complete body structures with attached wings (20 specimens). Deformations of some fossils by post-mortem transportation and other taphonomic factors are very discernible, especially on nearly complete specimens. Cui *et al*. (2011) have already documented the plastic deformations on grylloblattid insect specimens of Sinonamuropteridae from the same locality of the Tupo Formation.

The specimens were examined using Leica MZ12.5, Olympus SZX-9 and Nikon SMZ 645 stereomicroscopes in a dry state and under a film of ethyl alcohol. Line drawings were composed using a stereomicroscope and camera lucida attachment. Photographs were taken with a Canon D550 digital camera equipped with MP-E 65 mm and EF 50 mm macro-lenses in a dry state or under a film of ethyl alcohol. Original photographs were processed using the image-editing software Adobe Photoshop CS4, and some were processed by the stacking software Helicon Focus Pro.

Megasecopteran systematics follows Riek (1976) and Carpenter (1992) with modifications according to the concepts of Sinitshenkova (2002). Wing venation nomenclature generally follows the scheme of Kukalová-Peck (1991). Wing venation abbreviations: A1/A2—first/second anal vein, CuA/CuP—cubitus anterior/posterior, MA/MP—media anterior/posterior, RA/RP—radius anterior/posterior, ScP—subcosta posterior. Terminology for morphology of external genitalia was undertaken by Matushkina (2008) and Bauernfeind & Soldán (2012). Other abbreviations: bp—basal plate of ovipositor, ce—cerci, fc—forceps, pl—penis lobes (penes), V1/V2/V3—first/second/third valves of ovipositor, st—styliger (forceps base).

Systematic palaeontology

Superorder PALAEODICTYOPTEROIDA Bechly, 1996
Order MEGASECOPTERA Brongniart, 1885
Suborder EUMEGASECOPTERA *sensu* Riek, 1976
Family BRODIOPTERIDAE Carpenter, 1963 (monotypic)

Emended diagnosis. We follow the original diagnosis proposed by Carpenter (1963), with respect to the variability and specification of selected characters: MA either free or possibly connected to RP, CuA free or connected by a short crossvein to M, three or four simple anal veins, A1 and A2 diverging very close to the wing base.

Brodioptera Copeland, 1957

Type species. Brodioptera cumberlandensis Copeland, 1957; by original designation.

Brodioptera sinensis sp. nov. (Figs 1–9)

Etymology. Named after the the Latin name for China (Sina) where the holotype was found.

Diagnosis. (Based on forewing and hindwing venation). Wings elongate, nearly homonomous; hindwings slightly broader; crossveins simple, forming two parallel rows along posterior wing margin; costal margin slightly convex proximally with costal area enlarged basally; ScP ends at costal margin well behind midwing; RP pectinate with 3–4 terminal branches; simple MA connected for a short distance to RP just behind the separation of RA and RP; convex brace rp-m between veins RA+RP and M in basal part; MP and CuA simple; convex CuA connected by a short convex crossvein m-cua to M; CuP simple.

Holotype. CNU–NX1–600a, b (Figs 1, 2), (a) nearly complete imprint of well-preserved female, with head

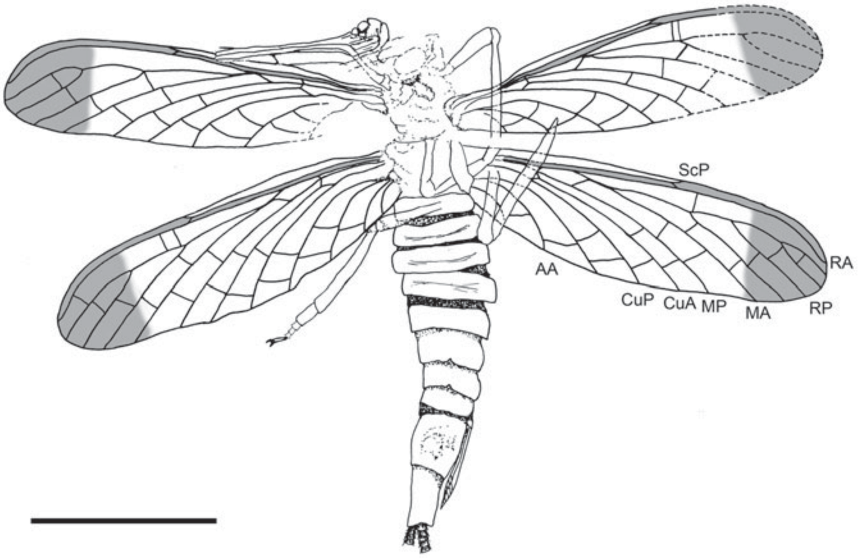

Fig. 1. Brodioptera sinensis sp. nov., holotype CNU–NX1–600a, b. Female: habitus line drawing (distal part of cerci omitted). Scale bar = 10 mm.

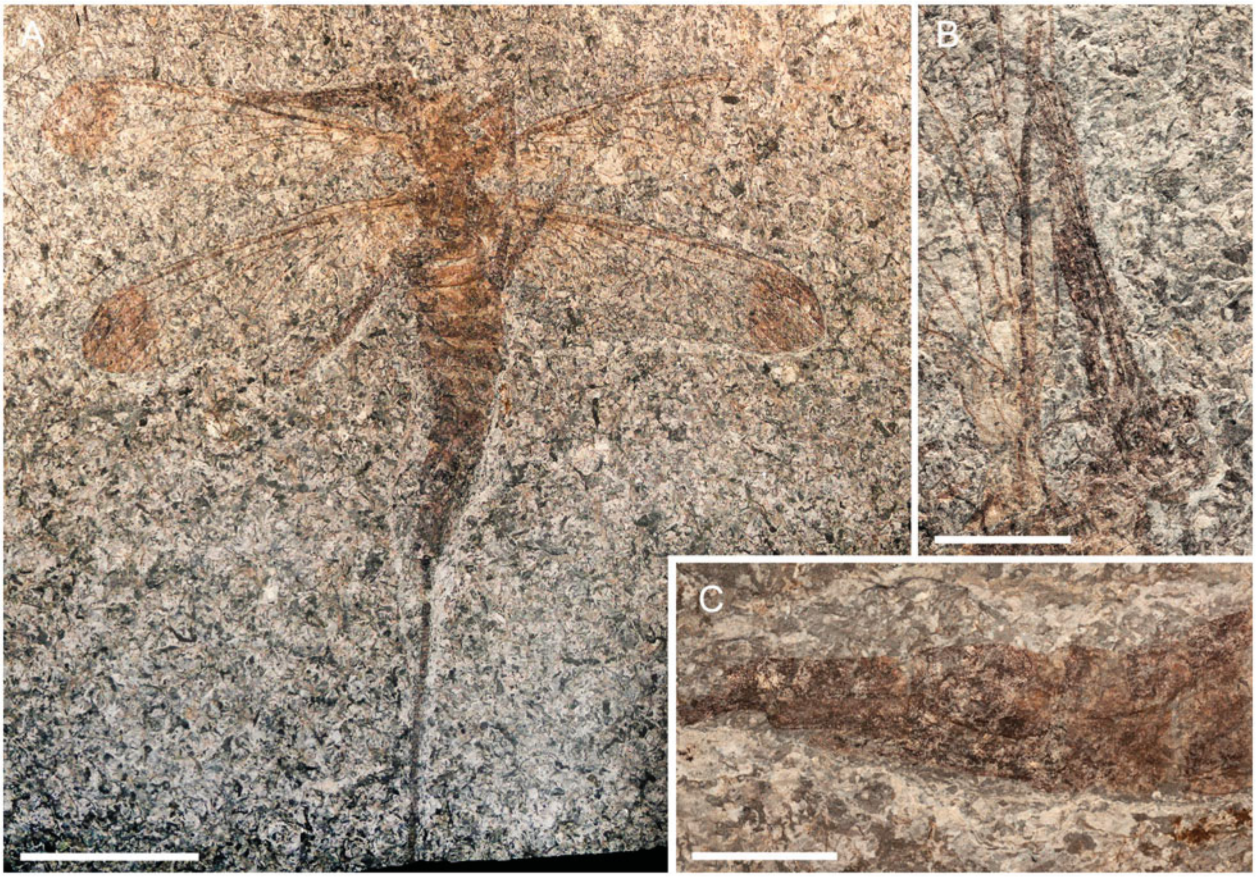

Fig. 2. Brodioptera sinensis sp. nov., holotype CNU–NX1–600b. Female: **A**, Photograph of habitus (imprint); **B**, detail of head in lateral position; **C**, detail of ovipositor (under alcohol). Scale bars A = 10 mm, B, C = 3 mm.

preserved, fragmentary thorax bearing well-preserved outstretched meso- and metathoracic wings, nearly completely preserved abdomen with ovipositor and a pair of incomplete multi-segmented cerci; (b) nearly complete counter-imprint of well-preserved female with body structures similar to the preservation of the imprint, ovipositor and cerci more complete.

Paratype. CNU–NX1–617 (Figs 3, 4, 7), nearly complete counter-imprint of well-preserved male with

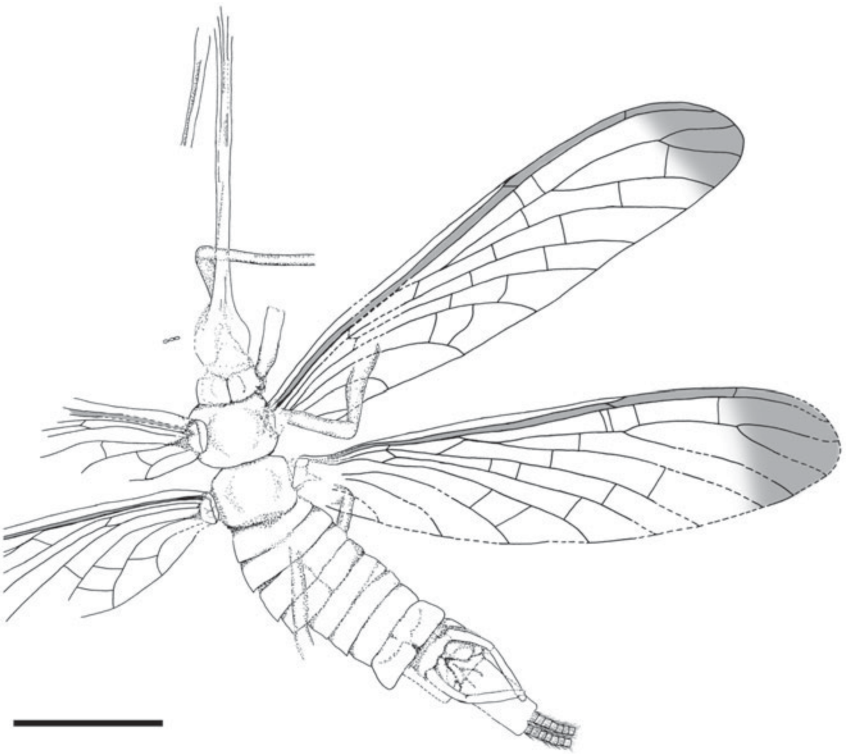

Fig. 3. Brodioptera sinensis sp. nov., paratype CNU–NX1–617. Male: habitus line drawing. Scale bar = 5 mm.

well-preserved head, thorax bearing one completely preserved forewing and other three wings preserved fragmentarily, complete abdomen with forceps and penes and fragmentary preserved pair of multi-segmented cerci.

Supplementary material

Body structures. CNU–NX1–652a, b, (female with preserved thorax, hindwings and nine abdominal segments with ovipositor), CNU–NX1–653a, b (male with partly preserved head, thorax, one hindwing, fragmentarily preserved legs and completely preserved abdomen with forceps and part of cerci), CNU–NX1–656a, b (specimen with fragmentarily preserved head, well-preserved thorax, proximal halves of four wings and proximal half of abdomen), CNU–NX1–659 (male with partly preserved thorax, one wing well preserved, two others only partially preserved, abdomen complete with forceps visible), CNU–NX1–601a, b (male with partly preserved head, thorax, one fore- and one hindwing, completely preserved abdomen), CNU–NX1–602a, b (completely preserved male with distal part of abdomen less visible but with more than half of total length of cerci preserved), CNU–NX1–605a, b (male with fragmentarily preserved thorax, legs and four wings, well-preserved abdomen), CNU–NX1–606a, b (completely preserved male with well-preserved thorax, fore- and hind wing), CNU–NX1–609a, b (Fig. 5), (completely preserved female with fragmentarily preserved thorax and four wings), CNU–NX1–610a, b (male with fragmentarily preserved head, thorax, legs, one fore- and one hind wing, completely preserved abdomen with forceps), CNU–NX1–613a, b (female with fragmentary head, thorax, legs, one wing and abdomen with ovipositor visible), CNU–NX1–614a, b (partially preserved specimen with thorax and apical parts of two wings), CNU–NX1–615a, b (specimen with well-preserved head, thorax, legs, only proximal parts of four wings and proximal half of abdomen), CNU–NX1–621a, b (Fig. 6), (specimen with well-preserved head with complete length of sucking mouthparts, prothorax, mesothorax preserved, metathorax only partly preserved, prothoracic legs visible, one wing completely preserved, other three only partially preserved), CNU–NX1–624a, b (distal half of wing and apical part of abdomen with ovipositor), CNU–NX1–632 (specimen with well-preserved head, thorax, legs and one fore- and one hind wing), CNU–NX1–635 (specimen with fragmentarily preserved head, thorax, four wings and proximal part of abdomen); CNU–NX1–651a, b (Fig. 5, 8), (female with well-preserved head, thorax and abdomen, wings barely visible).

Isolated wings. CNU–NX1–654a, b (complete wing), CNU–NX1–655 (apical two-thirds of wing), CNU–NX1–657a, b (nearly complete wing), CNU–NX1–658 (basal two-thirds of wing), CNU–NX1–660 (apical two-thirds of wing), CNU–NX1–661 (basal third of wing), CNU–NX1–662 (apical third of wing), CNU–NX1–663 (apical half of deformed wing), CNU–NX1–664 (basal third of wing), CNU–NX1–665 (apical third of

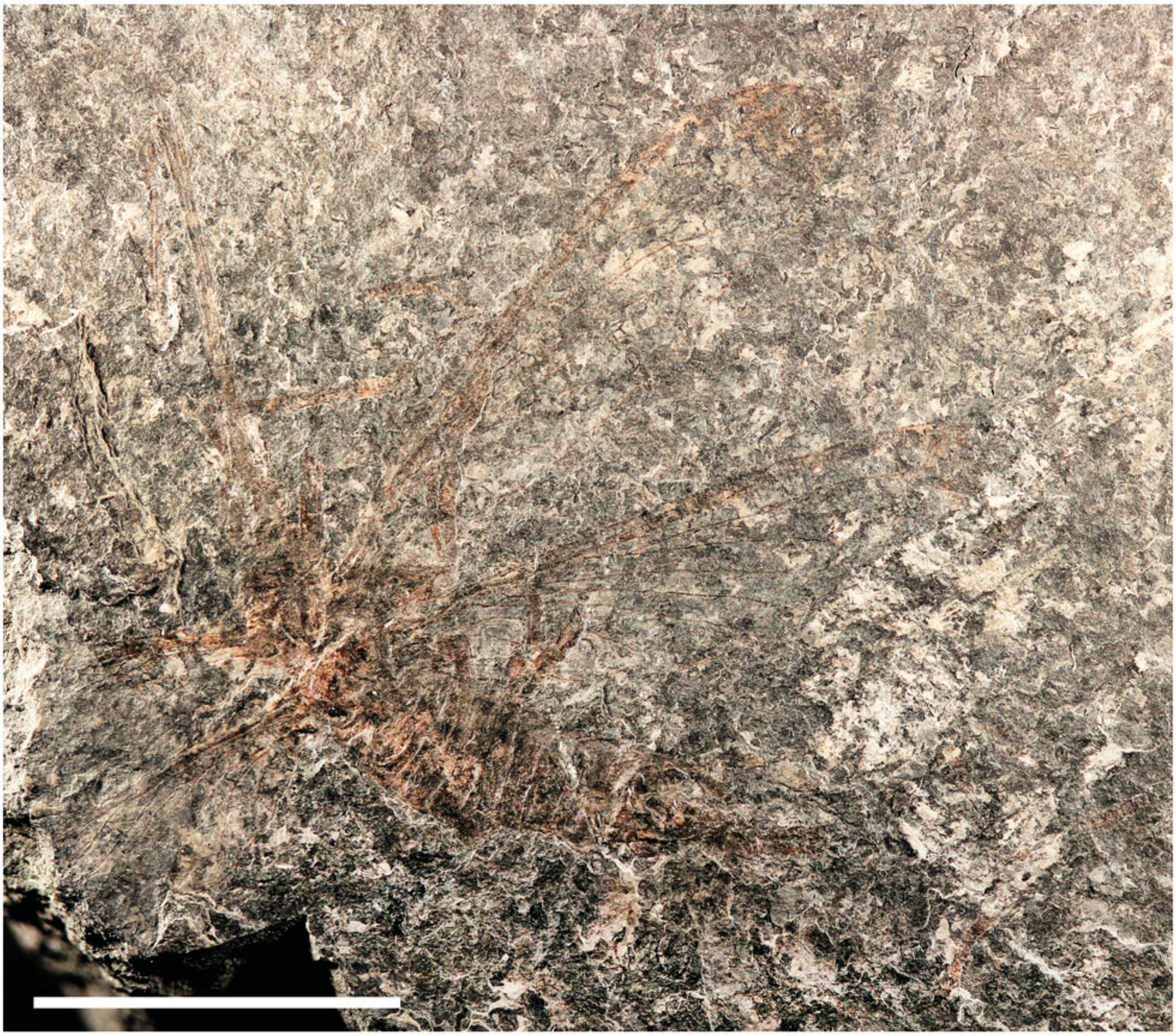

Fig. 4. Brodioptera sinensis sp. nov., paratype CNU–NX1–617. Male: Photograph of habitus (counter-imprint). Scale bar = 10 mm.

deformed wing), CNU–NX1–666 (apical half of deformed wing), CNU–NX1–603a, b (incomplete wing), CNU–NX1–604a, b (completely preserved wing with extensive deformation), CNU–NX1–607 (wing without basal part), CNU–NX1–608a, b (nearly complete wing with deformed apical part), CNU–NX1–611a, b (strongly deformed nearly complete wing), CNU–NX1–612a, b (completely preserved wing), CNU–NX1–616a, b (nearly complete and deformed wing), CNU–NX1–618 (basal third of wing), CNU–NX1–619 (complete basally deformed wing), CNU–NX1–620 (fragments of three wings), CNU–NX1–622a, b (basal half of wing), CNU–NX1–623a, b (apical two-thirds of wing), CNU–NX1–625 (apical half of wing), CNU–NX1–626 (complete, but basally deformed wing), CNU–NX1–627 (wing lacking apex), CNU–NX1–628a, b (wing lacking apex), CNU–NX1–629 (basal two-thirds of deformed wing), CNU–NX1–633 (apical two-thirds of deformed wing), CNU–NX1–634 (apical two-thirds of deformed wing), CNU–NX1–636 (apical half of wing), CNU–NX1–637 (complete slightly deformed wing), CNU–NX1–638 (complete slightly deformed wing), CNU–NX1–639 (fragments of four wings); all housed in the collections of Capital Normal University, Beijing, China.

Type stratum and locality. Tupo Formation, Upper Carboniferous, Bashkirian, (equivalent to Namurian B–C), Xiaheyan Village, Zhongwei County, Ningxia Hui Autonomous Region, China.

Descriptions. Holotype specimen No. CNU–NX1–600a, b (Figs 1, 2), female.

Caput: Relatively small hypognathous head bearing haustellate mouthparts with prominent elongated stylets reaching nearly half of wing size, triangular labrum and clypeus domed.

Thorax: Prothorax short, meso- and metathorax about the same size, legs with slender femora, long tibia

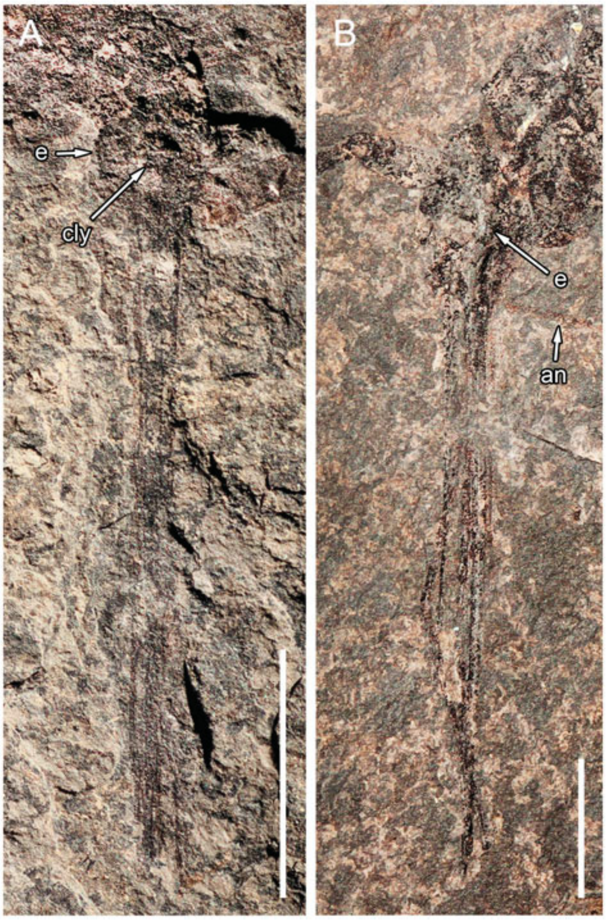

Fig. 5. Brodioptera sinensis sp. nov., **A**, Photograph of head CNU–NX1–609b; **B**, photograph of head CNU–NX1–651b. (e, eye; cly, clypeus; an, antena). Scale bars = 3 mm.

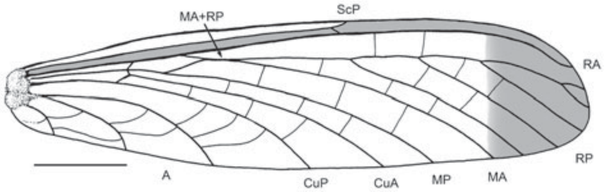

Fig. 6. Brodioptera sinensis sp. nov., CNU–NX1–621b. Line drawing of right forewing (imprint). Scale bar = 3 mm.

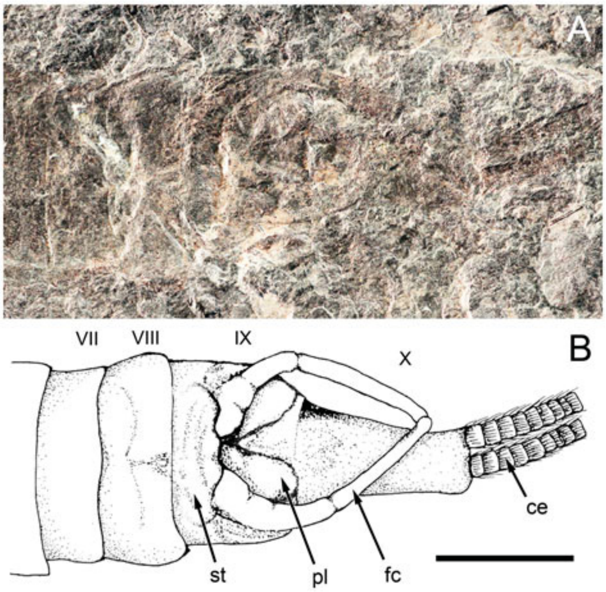

Fig. 7. Brodioptera sinensis sp. nov., paratype CNU–NX1–617. Male: distal part of abdomen bearing external genitalia. **A**, Photograph; **B**, line drawing [ce, cerci; pl, penis lobes (penes); st, styliger (forceps base); fc, forceps]. Scale bar = 2 mm.

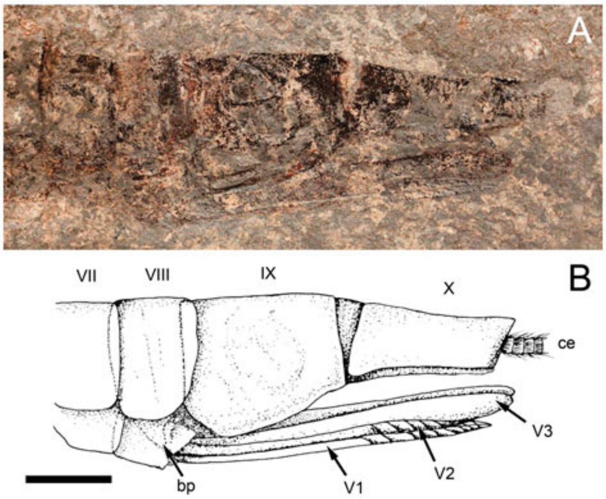

Fig. 8. Brodioptera sinensis sp. nov., CNU–NX1–651b. Female: distal part of abdomen bearing external genitalia. **A**, Photograph (under alcohol); **B**, line drawing (bp, basal plate of ovipositor; ce, cerci; V1/V2/V3, first/second/third valves of ovipositor). Scale bar = 2 mm.

with distinct patella, tarsus with five tarsomeres, last tarsomere with two tarsal claws. Wings nearly homonomous (hind wings slightly broader), both pairs markedly elongate, widest at about midwing, hyaline membrane with coloured apices, numerous simple straight crossveins between main longitudinal veins aligned in two rows; costal margin of the wing nearly straight, proximal part slightly convex, wing apex rounded, concave ScP straight reaching the costal margin just behind the midwing, short crossvein scp-ra present before the tip of ScP, area between ScP and RA darkly pigmented; radial and median veins running straight, closely parallel, division RA and RP about one-third of the wing length, RA simple reaching costal margin close to wing apex, RP pectinate ending with 3–4 terminal branches covering wing apex, convex MA diverges from MP close to the division of RA and RP, simple MA connected to RP just behind the separation of RA and RP for a distance of 0.7 mm, short convex brace rp-m present between veins RA+RP and M in basal part, concave MP simple, stem of Cu basally running parallel to M, division of CuA and CuP close to the wing base, simple convex CuA strongly diverges to M connected by a short convex crossvein m-cua aligned with rp-m, concave CuP simple, three or four simple anal veins forming reduced anal area.

Abdomen: Slender, widest in proximal part, gradually narrowing distally, with 10 segments, 9th and 10th abdominal segments markedly elongated, basal

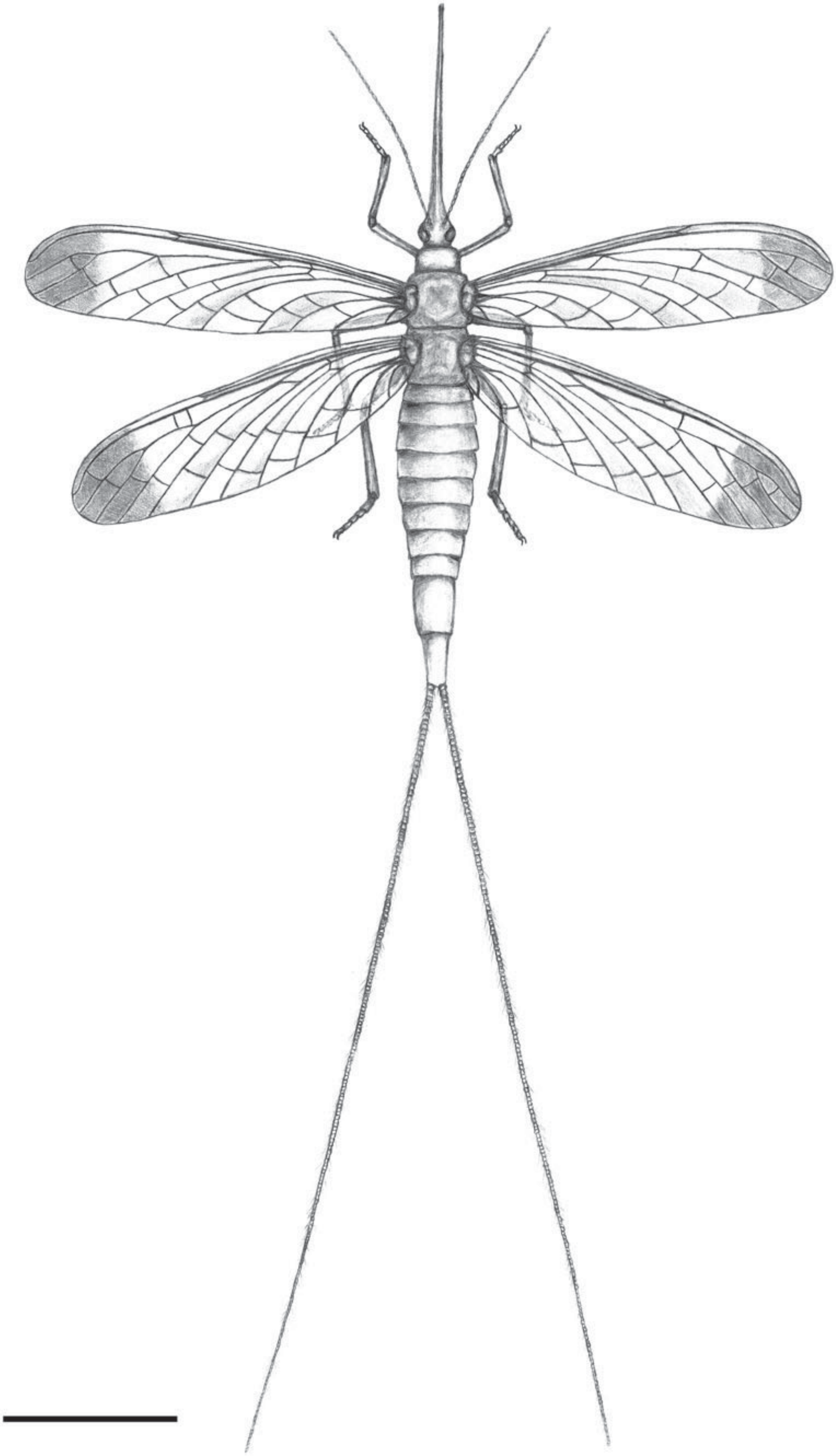

Fig. 9. Brodioptera sinensis sp. nov., Female: reconstruction of habitus based mainly on holotype CNU–NX1–600a,b and CNU–NX1–621a,b (head, antenae, prothorax and legs), CNU–NX1–602a,b (estimated length of cerci). Scale bar = 10 mm.

(subgenital) plate of ovipositor (bp) situated on 8th segment, ovipositor strongly sclerotized reaching about two-thirds of terminal segment, first and second valvulae in the form of cutting ridge. Last segment (10th) bearing a pair of multi-segmented cerci covered with dense setae.

Paratype specimen No. CNU–NX1–617 (Figs 3, 4, 7), male.

Caput: Relatively small, hypognathous, haustellate mouthparts with long stylets extending to two-thirds of wing length.

Thorax: Prothorax small, rectangular; meso- and metathorax similar in size bearing each pair of nearly homonomous wings connected by strongly sclerotized articular plates, both pairs of wings in outstretched position at rest, prominent median line clearly present on prothorax. Wings with hyaline membrane darkly coloured on wing apices about one-sixth of the wing length, basal part of wings with irregularly delimited, darkly pigmented membrane, wings markedly elongate, widest at about midwing, several simple crossveins present between main veins; costal margin nearly straight, concave ScP straight, reaching the costal margin well behind the midwing, short crossvein scp-ra present close to the tip of ScP, area between ScP and RA darkly pigmented, division RA and RP about one-third of the wing length, RA simple reaching costal margin close to wing apex, RP pectinate ending with four terminal branches covering wing apex, convex MA diverges from MP basad of the division of RA and RP, simple MA connected to RP close to the separation of RA and RP for a distance of 1.1 mm (right hindwing), concave MP simple, stem of Cu basally running closely parallel to M, division of CuA and CuP 2.7 mm from wing base (right hindwing), simple convex CuA diverges strongly to M connected by a short convex crossvein m-cua, concave CuP simple, three simple anal veins forming anal area.

Abdomen: Slender, distally tapering with 10 segments, 9th sternum present as subgenital plate (forceps base or styliger) and a pair of lateral forceps (styli) having two distinct segments not exceeding tip of abdomen, two penis lobes basally stout. The 10th segment partly preserved with elongated pair of multi-segmented cerci having prominent dense setation.

Dimensions. Holotype CNU–NX1–600a, b: Length of head including incomplete mouthparts 9.4 mm, thorax length 8.1 mm, width 3.5 mm; forewing length 21.0 mm, maximum width 5.3 mm, hindwing length 20.5 mm, maximum width 6.0 mm, wingspan 46.4 mm; abdomen length 18.3 mm, maximum width 5.0 mm, total body length 28.2 mm. Paratype CNU–NX1–617: Length of head including incomplete mouthparts 11.6 mm, thorax length 4.7 mm, width 2.6 mm; forewing length 18.7 mm, maximum width 4.3 mm, hindwing length 18.2 mm, maximum width 4.6 mm, estimated wingspan 45.8 mm; abdomen length 11.3 mm, maximum width 3.9 mm, total body length 21.2 mm.

Discussion. According to the key of Riek (1976), the present fossils are attributable to Brodiopteridae (Eumegasecoptera) based on the costal area being relatively broad, the wings having few straight crossveins, and ScP ending at the costal margin well beyond the midwing. Carpenter (1963) separated *Brodioptera* Copeland, 1957 from Brodiidae and established the monotypic family Brodiopteridae based on a broad wing base and costal margin lacking serration as the main diagnostic characters. Furthermore, the combination of the following venation characters strongly support the attribution to *Brodioptera*: (1) anterior margin nearly straight or slightly curved, (2) ScP ending on costal margin about midwing, (3) RP pectinate with several branches, (4) veins MA, MP, CuA and CuP simple.

Brodioptera comprises two described species, i.e., *B. cumberlandensis* Copeland, 1957 known from the Bashkirian (Namurian B) of Nova Scotia, Canada and *B. stricklani* Nelson & Tidwell, 1987 from the Bashkirian (lowermost Namurian B) of Manning Canyon, Utah, USA. *Brodioptera sinensis* sp. nov. shares with both latter species the shape of the wings with rounded apices, costal area relatively broad distally narrowing and relatively similar size of body and wings (Figs 1, 3, 6, 9). *Brodioptera sinensis* sp. nov. differs from both species by the presence of prominent connections of MA to RP and CuA connected to M for a short distance, and the presence of a short basal convex crossvein rp-m. However, we assume based on re-examination of *B. stricklani* that vein MA is shortly connected to RP by a crossvein on the right hind wing and omitted by the authors owing to its poor state of preservation (see Fig. 10). Wing length of *B. sinensis* sp. nov. ranges between 17 and 24 mm, and width between 4 and 6 mm, which corresponds to the dimensions of *B. cumberlandensis*. In addition, both previously described species lack any wing coloration. *Brodioptera sinensis* sp. nov. has predominantly darkly pigmented wing apices on both pairs of wings, but rarely a hyaline membrane (specimen Nos. CNU–NX1–605a, b: male, CNU–NX1–609a, b: female and CNU–NX1–632). We suspect that this phenomenon reflects freshly emerged imagoes or subimagoes without fully developed coloration or an aberrant character reflecting intra-specific variability of this species. Similarly, certain types of intra-specific variability are evident with respect to RP branches – these ranging from three to four equally represented on specimens. The presence of the first branch of RP being terminally twigged is rather scarce. The first anal vein is usually simple, but rarely terminally twigged (e.g., CNU–NX1–657a, b). Considering the relatively small differences in wing morphology, such as the distance of termination of vein

Fig. 10. Brodioptera stricklani, holotype No. 3160 Brigham Young University paleontological collection. Male: photograph of habitus (arrow indicates position of crossvein rp-ma). Scale bar = 10 mm.

ScP from the wing base to the costal margin between *B. cumberlandensis* and *B. stricklani*, these could fall easily into the variability of a single taxon. Note that the intra-specific variability of wing venation has already been assessed on other members of Palaeodictyopteroida, such as *Dunbaria fasciipennis* Tillyard in Dunbar & Tillyard, 1924 (Spilapteridae) and *Homoioptera vorhallensis* Brauckmann & Koch, 1982 (Homoiopteridae) (Kukalová-Peck 1971, Brauckmann 1991).

Our comparison also revealed a strikingly similar pattern of venation with darkly pigmented wing apices in *Sylvohymen pintoi* Brauckmann *et al*., 2003 (Bardohymenidae) known from Namurian of Hagen-Vorhalle, Germany (see Fig. 11). First, *S. pintoi* can not be placed in Bardohymenidae owing mainly to its broad wing base, wide costal area and the presence of at least two simple anal veins instead of single richly pectinate anal vein as in all Bardohymenidae. Therefore, we consider the placement of *S. pintoi* as erroneous and propose its transfer to *Brodioptera* (Brodiopteridae) as *Brodioptera pintoi* comb. nov. based on its possession of basally broad wings with a wide costal area, ScP ending beyond midwing and anal area with two simple veins.

Fig. 11. Brodioptera pintoi comb. nov., holotype P 21650 WMfN Westfälisches Museum für Naturkunde, Münster, photograph of forewing and hindwing. Scale bar = 10 mm.

Brodioptera pintoi (Brauckmann *et al*. 2003) shares with *B. sinensis* the pattern of coloration with dark wing apices and ScP ending close to the first branch of RP. It differs mainly in MA being shortly connected to RP. *Brodioptera pintoi* differs from *B. cumberlandensis* and *B. stricklani* by its more elongate wing shape with pointed apices. Nevertheless, this could be the result of deformations well known on fossils from this locality (see Ilger 2011). In future, this may result in the synonymy of *B. pintoi* with *B. stricklani*, but we prefer to retain separate taxa until further material is discovered.

Pinto (1986) described monotypic Xenopteridae with *Xenoptera riojanensis* from mid-Bashkirian (Namurian C) strata of the Malanzan Formation in Argentina sharing with *Brodioptera* mainly an enlarged costal area, ScP ending behind the midwing, and broad anal area formed by four veins. *Xenoptera* differs mainly in its branched MP, which is lacking in *Brodioptera*. Nevertheless, we suspect that Xenopteridae is probably closely related to Brodiopteridae particularly owing to their representatives' specialized anal area and similar age, despite their possibly different preferred climatic conditions (cool temperate vs tropical).

Ross *et al*. (2013) proposed emendation of Xenopteridae to Xenopteraidae for *Xenoptera* in order to remove homonymy of the family-group names Xenopteridae. Nevertheless, the International Commission on Zoological Nomenclature has not expressed any public opinion on the case (No. 3634), thus far. Although we support the proposal above, we employ Xenopteridae for *Xenoptera* in this study to avoid any subsequent misunderstanding.

Carpenter (1963) considered Brodiopteridae to be probably closely related to Corydaloididae and Sphecopteridae differing by the presence of partial connections between veins MA and RP, or these veins being shortly braced by crossvein rp-m, and partially connected veins CuA and M. However, both aforementioned families also differ from Brodiopteridae by the termination of ScP to RA or by having a free, markedly different anal area with pectinate anal veins. Therefore, we propose extending the diagnosis of Brodiopteridae (above) to include partial connections of veins MA and RP and the presence of crossvein between CuA and M. We also suspect that a crossvein between CuA and M is present in other species of *Brodioptera*, but unfortunately these parts are rather poorly preserved on the holotypes.

Another remarkable aspect concerning the functional morphology and elasticity in the basal part of wings is the step-like arrangement of crossveins rp-m and m-cua, together with basal divergence of CuA from CuP forming the possible basal reinforcement of the wing as previously noticed by Wootton & Kukalová-Peck (2000). This could have functioned similar to the extended anal brace present in some spilapterids, e.g., *Spilaptera spendens* Prokop *et al*., 2014 (Palaeodictyoptera; Prokop *et al*. 2014).

Morphology of head and mouthparts

The hypognathous head with haustellate type of mouthparts in the form of a beak with long stylets is a synapomorphy of groups within Palaeodictyopteroida. All members with sucking beaks were probably among the first specialized herbivores. Nevertheless, these structures are rarely preserved in megasecopterans, and therefore, our knowledge is rather limited. In spite of the rather poor state of preservation in most of the specimens of *B. sinensis* sp. nov., we provide evidence of at least several significant structures based mainly on supplementary material.

The small hypognathous head is probably triangular and bears elliptical eyes, filiform antennae reaching at least the tip of the stylets, a flagellum consisting of numerous antenommeres (Nos. CNU–NX1–602a, b; CNU–NX1–651), a prominent domed trapezoidal clypeus, and a triangular labrum (Nos. CNU–NX1–609, CNU–NX1–651). The most prominent characters are the extremely long and basally slender stylets, which are slightly longer than the forelegs, but the details of their morphology are not discernible. Maxillary palps are obviously segmented (No. CNU–NX1–651) but do not allow the restoration of their original segmentation (see Fig. 5). In contrast to *B. sinensis*, the mouthparts of other megasecopterans, such as *Protohymen*, or diaphanopterodeans, such as *Permuralia*, are distinctly shorter reaching at most about the middle of the forelegs (Kukalová-Peck 1974, fig. 11, Kukalová-Peck & Sinichenkova 1992, Rasnitsyn & Novokshonov 1997, figs 1–4). We note that these structures are virtually unknown in most taxa of both groups. Thus, haustellate mouthparts in the form of an extremely elongated slender beak, as present in *B. sinensis*, seem to be a rather unique adaptation among these groups signifying adaptation to specialized herbivory in Bashkirian ecosystems.

Morphology of external male and female genitalia

Male external genitalia are visible mainly on the paratype and supplementary specimens (Nos. CNU–NX1–653a, b, CNU–NX1–601a, b, CNU–NX1–605a, b, CNU–NX1–606a, b, CNU–NX1–610a, b) and are represented by a slightly enlarged forceps base (subgenital plate, styliger) and at least two-segmented slender forceps ending well before the tip of the abdomen. The penis consists of paired basally stout penes (two penial lobes). Nelson & Tidwell (1987) described the external male genitalia in the holotype of *B. stricklani* to consist of the lateral claspers as forceps similar in shape and size to *B. sinensis* sp. nov. and medial gonapophyses without visible segmentation owing to the poor state of preservation (see Fig. 10).

Carpenter (1939) described external male genitalia in *Permohymen schucherti* Tillyard, 1924 (Protohymenidae) as bearing a pair of claspers (=forceps) consisting of two segments strikingly similar to *B. sinensis* sp. nov. (see Fig. 12). The distal part of the forceps also exhibits marked setation and tubercles along the inner margin of the terminal segment. Nevertheless, the two penial lobes seem to be more slender than in *B. sinensis* sp. nov. Carpenter (1951) reported the presence of an obscure plate behind the 8th segment with a pair of processes (forceps) on *Aspidothorax triangularis* Brongniart, 1893 (Aspidothoracidae) similar to ones present in Protohymenidae. We concur that the forceps of Megasecoptera and recent Ephemeroptera are notably similar as indicated by Carpenter (1939) and Nelson & Tidwell (1987). Nevertheless, it seems that the location of the forceps base in Megasecoptera is distinctly more basal in comparison with extant Ephemeroptera. The segmentation of forceps in Ephemeroptera is rather variable usually with four or five segments, but rarely with a single segment (*Caenidae*) or segmentation completely missing (*Homoeoneuria* spp.); (Bauernfeind & Soldán 2012). Kukalová-Peck (2008, p. 27) noticed the similarity of the male external genitalia between the Permian diaphanopterodeans and modern ephemeropterans (Siphonuridae). The male external genitalia of *Permuralia maculata* (Kukalová-Peck & Sinichenkova, 1992) (Diaphanopterodea) also seem to be superficially similar to gonocoxae and gonostyli corresponding to two-segmented forceps in *B. sinensis*, but the presence or absence of two penial lobes can not be confirmed owing to poor preservation of this part (Kukalová-Peck &

Fig. 12. Permohymen schucherti Tillyard, 1924, No. MCZ 3819 Museum of Comparative Zoology, Cambridge, USA, detail of male external genitalia. Scale bar = 1 mm.

Sinichenkova 1992, Sinichenkova & Kukalová-Peck 1997, Rasnitsyn & Novokshonov 1997). Such male genitalia with two-segmented forceps also occur in *Asthenohymen uralicum* Zalessky, 1939 (Diaphanopterodea) (Novokshonov & Willmann 1999, p. 544).

Female external genitalia in the form of a heavily sclerotized ovipositor consist of a gonoporus located behind the 8th segment surrounded by the first pair of valvulae (V1) and the second pair of valvulae (V2). Apical parts of the first and second valvulae are laterally serrated in the form of a cutting ridge with 9–10 oblique ridges (V1) and about seven oblique ridges (V2). The basal plate present on CNU-NX1-651a, b is partly distorted. The third pair of sheathing valvulae (V3) are elongate and markedly broader than V1 and V2 to cover the ensheathing cutting valves in the resting position. The surface of V3 bears with obvious punctuation probably corresponding to the original setation. The apical stylus is not present. Such an architecture of the ovipositor corresponds to the endophytic type known in Megasecoptera, Diaphanopterodea, but also distantly Zygoptera (Odonata). Nevertheless, the zygopteran type of ovipositor differs mainly in that the enlarged V3 bears prominent denticles ventrally in the form of a carina and an apical stylus (see Matushkina & Lambret 2011). Such a prominent apical stylus is also present on *Permuralia maculata* but not in *B. sinensis* (Rasnitsyn & Novokshonov 1997, p. 33).

Thus far, the ovipositor of *Brodioptera* was unknown because the only nearly complete specimen of *B. stricklani* was a male. Novokshonov & Willmann (1999) described female external genitalia of *Asthenohymen uralicum* Zalessky, 1939 (Diaphanopterodea) as a slightly curved endophytic ovipositor lacking styli, which corresponds well to *B. sinensis* sp. nov. Moreover, the comparison of male external genitalia of *Asthenohymen uralicum* and *B. sinensis* consisting of two-segmented forceps and the base emerging behind the 8th segment supports their close relationship. A similar type of ovipositor also occurs in *Permohymen schucherti* (Megasecoptera: Protohymenidae) and *Asthenohymen dunbari* Tillyard, 1924 (see Fig. 13) as previously noted by Carpenter (1939). Other megasecopterans, such as *Foriria maculata* Meunier, 1908 have an endophytic ovipositor similar in size, but lacking discernible apical dentation perhaps owing to poor preservation (Béthoux *et al.* 2004). Nevertheless, we concur that there is a striking similarity of external genitalia between members of Megasecoptera and Diaphanopterodea.

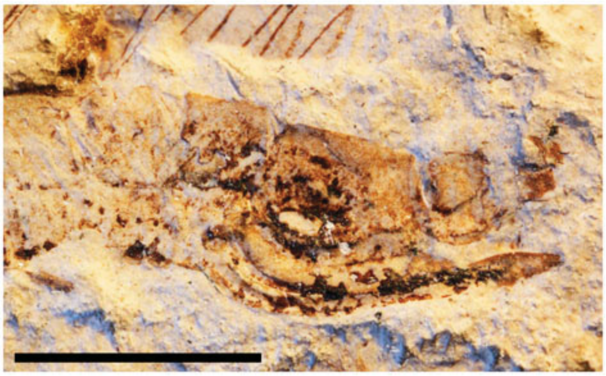

Fig. 13. Asthenohymen dunbari, Tillyard, 1924, No. MCZ 3059 Museum of Comparative Zoology, Cambridge, USA, detail of female external genitalia. Scale bar = 2 mm.

Conclusions

The first megasecopteran, *Brodioptera sinensis* sp. nov. (Brodiopteridae), described from the Early Pennsylvanian of the Tupo Formation in northern China allows resolution of intra-specific variability on the basis of 54 specimens. The new species is based on a distinctive wing venation pattern with apparent variability in coloration. The morphology of other body structures revealed markedly elongate haustellate mouthparts in the form of a beak, and external copulatory organs of both sexes that were previously unknown or poorly preserved. The female genitalia are in the form of an endophytic ovipositor with apical parts of the first and second valvulae laterally serrated and bearing oblique ridges, and the male styliger has long two-segmented forceps strongly resembling those of members of Protohymenidae (Megasecoptera) and Asthenohymenidae (Diaphanopterodea). Moreover, the male external genitalia seem to be similar to some taxa of extant mayflies (e.g., Siphlonuridae) differing in their more basal position. We emend the diagnosis of the monotypic Brodiopteridae. We consider Brodiopteridae to be closely related to Xenopteridae, owing to their similar wing venation (especially the specialized anal area) and also their consistent Bashkirian age despite their occurrence in different palaeoclimates (cool temperate vs tropical). Based on our re-examination of the material, we also propose transferring *Sylvohymen pintoi*, known from Namurian of Hagen-Vorhalle (Germany), to Brodiopteridae as *Brodioptera pintoi* comb. nov. The occurrence of *Brodioptera sinensis* sp. nov. and other brodiopterids supports the hypothesis of faunal exchange between Euramerica and North China during the Bashkirian.

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Článek 3

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Hidden surface microstructures on Carboniferous insect *Brodioptera sinensis* (Megasecoptera) enlighten functional morphology and sensorial perception

Jakub Prokop[1], Martina Pecharová[1] & Dong Ren[2]

Megasecoptera are insects with haustellate mouthparts and petiolate wings closely related to Palaeodictyoptera and one of the few insect groups that didn't survive the Permian-Triassic mass extinction. Recent discovery of *Brodioptera sinensis* in early Pennsylvanian deposits at Xiaheyan in northern China has increased our knowledge of its external morphology using conventional optical stereomicroscopy. Environmental scanning electron microscopy (ESEM) of structures, such as antennae, mouthparts, wing surfaces, external copulatory organs and cerci have shed light on their micromorphology and supposed function. A comparative study has shown an unexpected dense pattern of setae on the wing membrane of *B. sinensis*. In addition, unlike the results obtained by stereomicroscopy it revealed that the male and female external genitalia clearly differ in their fine structure and setation. Therefore, the present study resulted in a closer examination of the microstructure and function of previously poorly studied parts of the body of Paleozoic insects and a comparison with homologous structures occurring in other Palaeodictyopteroida, Odonatoptera and Ephemerida. This indicates, that the role and presumptive function of these integumental protuberances is likely to have been a sensory one in the coordination of mouthparts and manipulation of stylets, escape from predators, enhancement of aerodynamic properties and copulatory behaviour.

Megasecoptera is a small group of Late Paleozoic phytophagous insects having haustellate type mouthparts in the form of a rostrum with elongated stylets and permanently outstretched basally narrow wings with corrugated longitudinal veins and generally few cross-veins. This group belongs to the extinct Palaeodictyopteroida, are uncertain systematic position and either an ingroup of Palaeoptera or sister-group of Neoptera[1–3]. The family Brodiopteridae are restricted to the Namurian stage with one of its members, *Brodioptera stricklani*, the oldest Megasecoptera and at the same time one of the earliest winged insects (Pterygota) recorded close to the boundary between Mississippian and Pennsylvanian[4]. Recent discovery of the genus *Brodioptera* in Early Pennsylvanian deposits in China provides evidence for faunal exchange between Euroamerica and Northern China during the Bashkirian[5]. *Brodioptera sinensis* was described on the basis of a well preserved series of specimens in various aspects of preservation, which revealed intra-specific variability in wing venation[6]. Moreover, the reconstructed species shows details of its morphology, like haustellate mouthparts with conspicuous elongated stylets, wings with a well preserved pattern of venation and male and female external genitalia that were previously poorly documented or unknown in these insects (see Fig. 1). Therefore, this material offers an excellent opportunity for a detailed study of the micromorphology of certain structures using ESEM and to use this information to determine the likely function of similar structures in related taxa.

Scanning electron microscopy (SEM) has been used to study surface microstructures of arthropods for almost half a century. However, it is rather rarely used for studying Paleozoic insect fossils, with a few exceptions, such as

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Figure 1. *Brodioptera sinensis*, adult female, reconstruction of habitus based on series of the specimens as resting on sphenophyte stem *Calamites* sp. (Calamitaceae), wing span cca 46 mm, Late Carboniferous, China (drawn by MP).

searching for pollen grains or spores in their gut contents, studying the micromorphology of delicate structures, chaeototaxy and phoretic organisms[7–10]. The poor state of the majority of imprint fossils damaged by postmortal deformations makes their study particularly difficulty and often end in failure. These techniques have been more widely used in studies of fossils from younger strata, such as Mesozoic and Cenozoic amber inclusions and exceptionally preserved compressed fossils[11–14]. The broader application to the study of scarce insect fossils was accelerated recently with use of ESEM, which makes it possible to study uncoated specimens using this non-invasive technique[15].

Thus, this study presents a more detailed and clearer view of surface microstructure of certain parts of the body of a megasecopteran, *B. sinensis,* using the extensively and well documented material from the Late Carboniferous in northern China[6].

Results and Discussion

Head structures, in particular antennae and mouthparts. The head is hypognathous, relatively small and triangular in shape with elliptical bulging eyes (ce) (Fig. 2a). Antennae filiform, generally incompletely preserved and nearly reaching the tip of the rostrum (CNU–NX1–602a), with enlarged scape (sc) and short pedicel (pe), both poorly delimited and a long multi-segmented flagellum consisting of short elliptical flagellomeres (fl) (Fig. 2b). These long antennae were either held wide spread in flight, which would greatly reduce their air speed, or positioned closely along the sides of the rostrum as in several specimens in which these structures are fossilized. Mouthparts of haustellate type with a rostrum; the beak, consist of a pair of slender long markedly sclerotized mandibular stylets (md) (Fig. 2b,c) and paired less sclerotized stout multi-segmented maxillary palps (mp) basally connected to the maxillae underneath the md stylets, the microstructure on consists of scattered sensory setae on up to 180 μm of their length (Fig. 2c,d). This insect probably used these setae as mechanoreceptors for coordinating its mouthparts and manipulation of stylets while feeding. The two pairs of aforementioned stylets dorsally cover the labium (lb) that consists of a pair of lobes (Fig. 2c). The head capsule is usually distorted due to taphonomy, but a triangular labrum (lm) and poorly preserved domed trapezoidal clypeus (cl) can be distinguished (Fig. 2a,b).

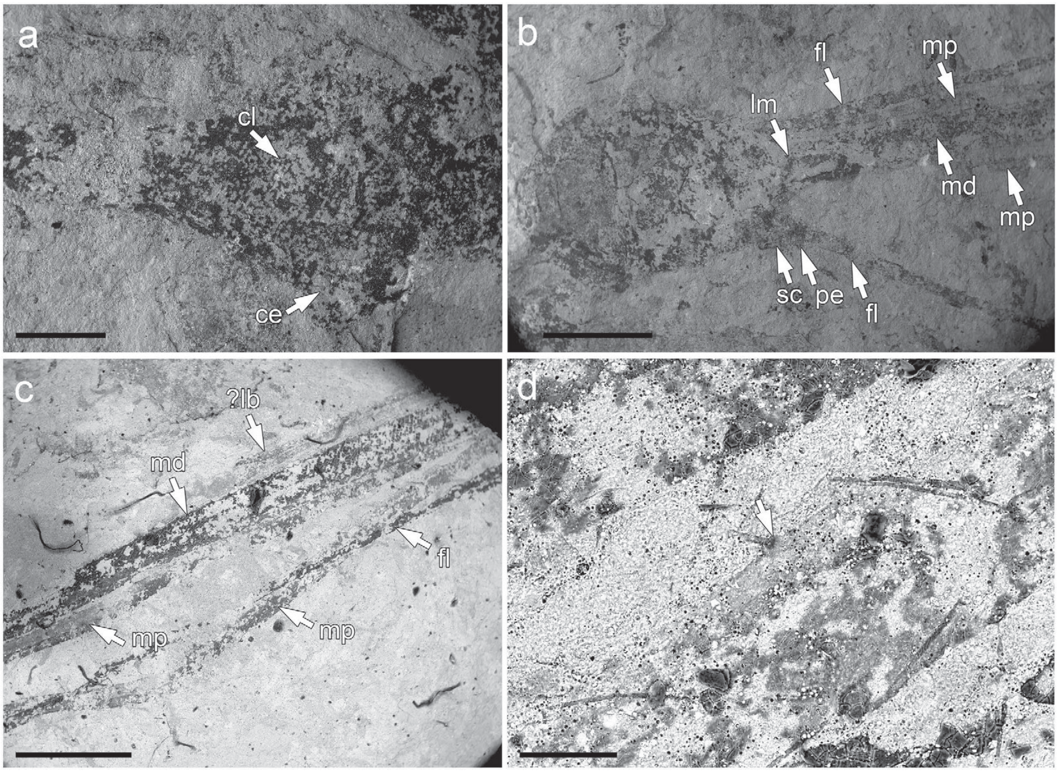

Figure 2. ***Brodioptera sinensis*, scanning electron micrographs of head structures, Capital Normal University, Beijing, China.** (**a**) Surface of CNU–NX1–609 (**b**) CNU–NX1–602 (**c,d**) stylets with close ups surface of setae CNU–NX1–651. Arrows indicate ce - compond eye, fl - flagelum, lb - labrum. Scale bars 500 μm (**a**), 1000 μm (**b,c**), 100 μm (**d**).

This type of unique mouthparts with a prominent rostrum is considered as a synapomorphy for members of the Palaeodictyopteroida, which includes the orders Palaeodictyoptera, Megasecoptera, Permothemistida and Diaphanopterodea[1,2,4,16]. However, the mouthparts of these fossils are poorly preserved and hence only a few taxa have been studied in detail, for instance the diaphanopterodean *Permuralia maculata* known from the Early Permian in Russia[16–19], and palaeodictyopteran *Eugereon boeckingi* (Eugereonidae) known from the Late Carboniferous in Germany and *Monsteropterum moravicum* (Homoiopteridae) from the Early Permian in the Czech Republic[20,21] among others. The mouthparts of *B. sinensis* seem to correspond particularly well with those of *P. maculata* in having a distinctly shorter triangular labrum, a pair of closely aligned strongly sclerotized mandibulary stylets and broad and weakly sclerotized maxillary stylets. The rostrum of *B. sinensis,* however, is markedly longer than that of *P. maculata*, which may indicate it fed on different type of plant. Interestingly, another megasecopteran, with a relatively short and stout beak, *Sinopalaeopteryx olivieri* (Aykhalidae) is also known from Xiaheyan, which is evidence of the evolution of different types of herbivory in this group of insects during the Bashkirian[22]. In this context, it is noteworthy that specific piercing and sucking damage caused by the stylets of Palaeodictyopteroida to marattialean tree ferns and to Cordaite seeds are recorded[16,23–25]. Nevertheless, such record from Xiaheyan locality has not been reported so far.

Material examined: Nos. CNU–NX1–600a, b (holotype); CNU–NX1–617 (paratype); CNU–NX1–602a,b; CNU–NX1–609a,b; CNU–NX1–615a,b; CNU–NX1–621a,b; CNU–NX1–632; CNU–NX1–651a,b.

Wing surface structures. The shape and poorly developed wing musculature of the wings of Megasecoptera indicate that they were adapted for slow flight or even hovering[26]. The wing membrane of *B. sinica* is covered by irregularly scattered setae with a few also on the veins. There is a marked decrease in the density of setae from the base to the apex of a wing. All these setae, are on basal circular sockets (Fig. 3c,d), nearly straight and structurally similar, with a maximum size of about 100 μm and, therefore, considered to be macrotrichia (Fig. 3a–d). They probably functioned as mechanoreceptors. The surface structures on insect wings, such as small bristles, scales and setae are widely studied in recent insects, especially in terms of their association with flight ability. These structures are active during flight in members of the Odonata, Diptera, Hymenoptera and Lepidoptera[27–29]. The setae on the wing membrane cause microturbulences and possibly decrease friction during flight by creating a boundary layer between the air stream and wing surface[30]. That is, these setae could provide a flying insect with enhanced lift and reduced drag, or alternatively the setae are hydrophobic as they are much longer and more clustered in teneral adults or potentially subimago than in the adults. Similar structural differences in microstructure of wings of adult and subimago modern mayflies are reported by several authors[31]. However, these setae on the wing membrane of modern Ephemeroptera are microtrichia and distinctly smaller in size. Thus, the suggestion

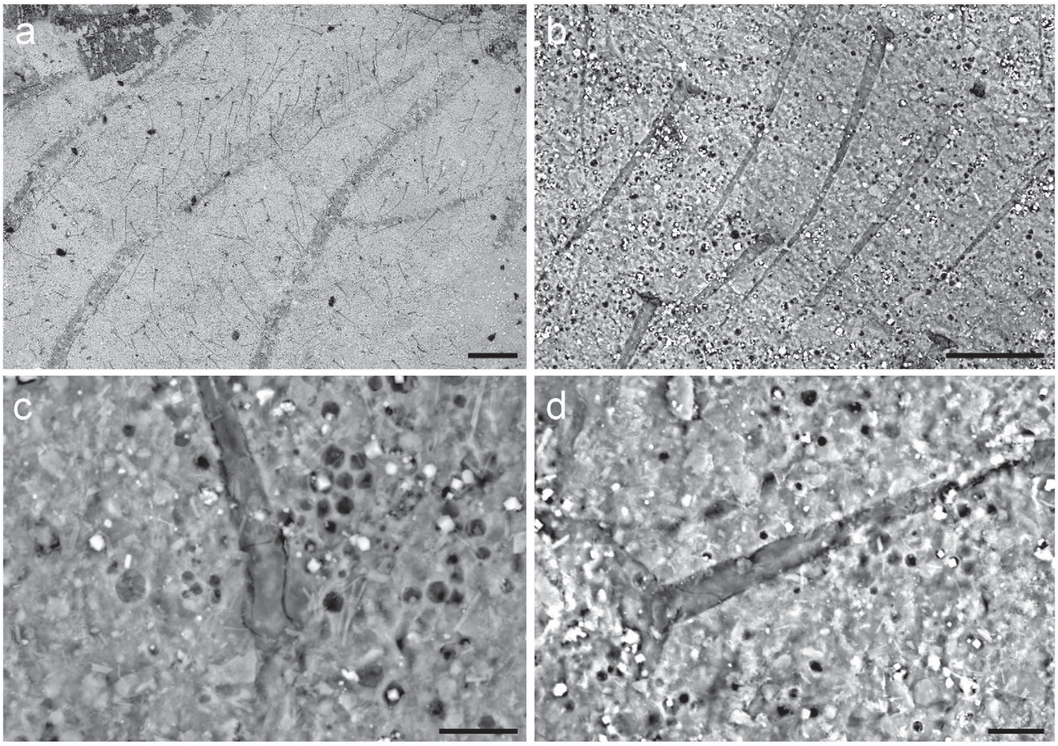

Figure 3. ***Brodioptera sinensis*, scanning electron micrographs of wing structures, Capital Normal University, Beijing, China.** (**a**) Surface of hindwing anal area with scattered setae CNU–NX1–632. (**b–d**) Detail of setae CNU–NX1–632. Scale bars 200 μm (**a**), 50 μm (**b**), 10 μm (**c,d**).

that the function of these structures is hydrophobic (microtrichia) in megasecopterans is not well supported. Particularly, because it implies an aquatic lifestyle of the nymphs of these insects, which is currently not widely accepted, and supports the original idea[32–34]. The record of their immature stages is rather poor and available specimens entirely known from the Carboniferous ironstone nodules as members of Mischopteridae do not show the mentioned micro structural details which could enlighten their lifestyle unambiguously[35]. The specialized type of mouthparts of nymphs and their articulated wing pads in slightly expanded position from the thorax support hypothesis that they had a similar diet and habitat preference as adults. Nevertheless, it is not possible to exclude the possibility that *B. sinica* had two types of setae on its wings, as is the case in modern lacewings, like *Micromus tasmaniae* (Hemerobiidae), and the tiny microtrichia cannot be observed due to taphonomy[36] (Figs 3 and 4).

Our observations on wing surface microstructure of various specimens of *B. sinica* indicates two different stages: a teneral adult or potentially subimago (CNU–NX1–605, CNU–NX1–609, CNU–NX1–632) with wings without darkly coloured apices, which are often creased with distinctly longer and clearly more densely clustered macrotrichia, possibly having a hydrophobic function, and an imago with darkly coloured wing apices, setae on wing membrane distinctly shorter and sparsely clustered. Moreover, this is supported by females with the ovipositor in the teneral adult or subimago stage slender and always hidden in the third pair of valvulae, while in the imago the ovipositor is broad and the first and second pair of valvulae preserved in an exposed position and separate from the third pair of valvulae.

The wing membrane of Megasecoptera is generally hyaline with rarely any well-developed macrotrichia as in members of the family Bardohymenidae[37]. The venation of *Actinohymen russelli* (Bardohymenidae) bears setal sockets in rows on the main longitudinal veins CA+CP, ScP and R[38]. Prominent serrations or knob-like elliptical protuberances (tubercles) are also documented on the veins on the anterior wing margin of Brodiidae, as in *Brodia priscotincta* and *Eubrodia dabasinskasi*[39]. Bolton considers these spinules to be modifications of long hairs[40], "macrotrichia", which supports the view of Tillyard[41]. Corresponding structures are known in other members of Palaeodictyopteroida (e.g., Anchineuridae, Namuroningxiidae (see Fig. 4a), Protohymenidae) and also in some members of recent insects, like Odonata, etc[42–44]. Moreover, there are prominent spines projecting apically on the basal part of the margin of the hind wing in Brodiidae[39]. Kukalová-Peck records the common presence of macrotrichia on wings of members of Palaeodictyoptera[45], nevertheless, the evidence for this is weak. Macrotrichia on the membrane are not widespread among megasecopterans, as demonstrated for *Namuroptera minuta* (Aykhalidae, see Fig. 4b), which is known from the same locality as *B. sinensis* and lacks such setae on its wing membrane[22]. Hence, this disparity in wing surface microstructures in Megasecoptera was present since at least the Late Carboniferous and their function is probably related to their flight ability rather than hydrophobic.

Material examined: CNU–NX1–600a,b (holotype); CNU–NX1–601a,b; CNU–NX1–605; CNU–NX1–609a,b; CNU–NX1–621a,b; CNU–NX1–632.

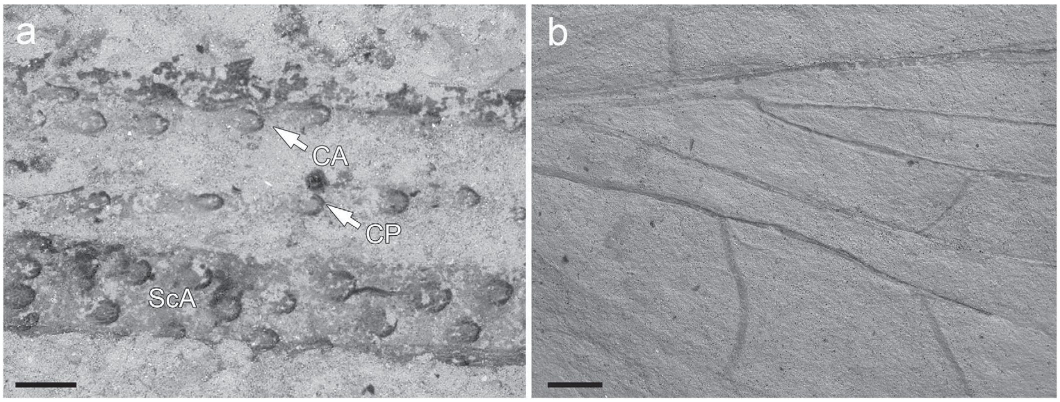

Figure 4. ***S**canning electron micrographs of wing structures, Capital Normal University, Beijing, China.* (**a**) *Namuroningxia elegans* (Namuroningxiidae) with knob like tubercles located on the veins CA, CP and ScA, CNU–P–NX2006001. (**b**) *Namuroptera minuta* (Aykhalidae), surface of wing basal part CNU–NX1–646. Scale bars 200 μm (**a,b**).

Abdomen, in particular external genitalia, cerci and their microstructures. External copulatory organs of Carboniferous insects are rarely recorded especially for both sexes in several specimens. Therefore, their fine structure revealed by ESEM is described in this section. The abdomen of *B. sinica* is 10-segmented with apex bearing a pair of long multi-segmented and rather stout cerci. The cerci are covered with prominent protruding setae up to 500 μm long arranged in rings along the posterior edge of each segment. We think that these setae are trichoid sensillae, which are common in modern insects and most probably function as tactile sensory setae or air movement receptors for control of yaw stability in flight (see Fig. 5). It is likely that these setae enable them to detect the lunging movement of a predator and immediately escape by running or flying away. Novokshonov and Willmann describe segmented basally stout cerci bearing long setae arranged along the posterior margin in the diaphanopterodean *Asthenohymen uralicum* (Asthenohymeniodae) from the Early Permian in Chekarda in the Central Urals[46].

Male external genitalia consist of enlarged forceps base (styliger, st) with the slender posteriorly situated forceps (fc) curving distally (Figs 6 and 7b). Forceps are at least two segmented and reach the tip of the abdomen as was described in our previous study, but re-examination of specimen CNU–NX1–601b using ESEM revealed the possible presence of a short additional terminal segment (Fig. 6b). Penis clearly consists of two penial lobes (pl) slightly enlarged basally and straight for sperm transfer (Fig. 6a,c). The ESEM study also indicates the apices of penial lobes are more slender (possibly titillator processes) than reported in our previous study using light stereomicroscopy[6]. It is likely that there are setae on the surface of the penial lobes and a cluster of terminal setae at their apices (Fig. 6c) but we are unable to confirm this due to processes that occurred during taphonomy. Homologous structures are recorded in *Permohymen schucherti* (Protohymenidae) from the Lower Permian in Kansas[47], which bear a pair of two segmented claspers (forceps) and two penial lobes that are strikingly similar to those of *B. sinensis*. However, on the basis of our re-examination of specimen MCZ 3819b these penial lobes are distinctly shorter and have stouter terminal appendages than *B. sinensis*. The forceps (gonostyli) are covered with tactile setae that are probably mechanoreceptors, however their distal segments bear densely clustered setae. Another brodiopterid, *B. stricklani*[48], known from the Bashkirian in North America has male genitalia that are very similar with the lateral elongated claspers acting as forceps, but a closer comparison of their segmentation is not possible because of their poor state of preservation. Furthermore, our re-examination of Permian *Protereisma permianum* (Permoplectoptera: Protereismatidae) and *Misthodotes obtusus* (Permoplectoptera: Misthodotidae) revealed that the structures of male external genitalia bearing the enlarged forceps basis with a pair of five segmented forceps pointed apically and elongated slender apices of the penial lobes are homologous[49]. Thus, this study of morphology, including surface microstructures, confirms that the external male copulatory organs of Megasecoptera and Ephemerida are very similar as previously suggested[48,50] and others. Furthermore, our comparison was of several well documented members of the order Diaphanopterodea in which the structures of the external male genitalia and other body characters are homologous, but at rest the wings of which are positioned along the abdomen. The best studied species is *P. maculata*, for which the male external copulatory organs are reconstructed and interpreted differently[17,19]. Our comparison is supported by the re-examination of series of specimens adopting the more conservative view[19]. In the poorly preserved *Permuralia* the corresponding structures are two segmented forceps (gonocoxae and gonostyli) and well separated and straight penial lobes. Similarly, male external copulatory structures bearing long two segmented forceps with tubercles on inner part of the gonostyli are also described in *Asthenohymen uralicum*. Finally, the comparison of *B. sinensis* to the corresponding primary male copulatory structures known in *Namurotypus sippeli* (Meganisoptera: Namurotypidae) as one of early diverging group of Odonatoptera revealed the presence of a pair leaf-like segmented gonopods and paired penial lobes which are regarded as synapomorphy of Palaeodictyopteroida, Ephemeropterida and Odonatoptera[51]. Considering the length of distal abdominal segments and position of forceps in *B. sinensis* in contrast to extant mayflies we assumed that high flexibility of these segments allowing copulatory position. Interestingly, the hypothesis of indirect copulation behavior and deposition of spermatophores as proposed for

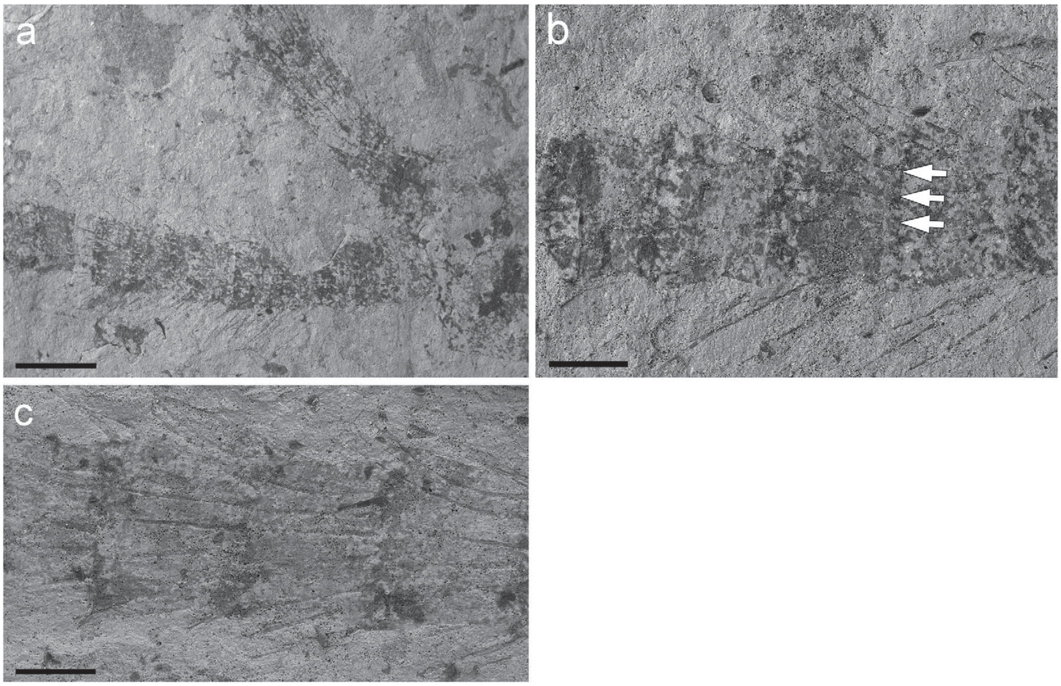

Figure 5. ***Brodioptera sinensis*****, scanning electron micrographs of cerci, Capital Normal University, Beijing, China.** (**a**) Apex of abdomen with basal part of cerci CNU–NX1–602a. (**b**) Detail of cerci with protruding setae CNU–NX1–602a. (**c**) Detail of cerci with protruding setae CNU–NX1–600b. Arrows indicate bases of setae surround the posterior edge of segments. Scale bars 500 μm (**a**), 200 μm (**b,c**).

Namurotypidae by Bechly[51] seems to be unlikely for *Brodiptera* due to striking resemblance of distally pointed forceps with modern mayflies and also the presence of clustered sensory structures.

Material examined: CNU–NX1–617 (paratype); CNU–NX1–601a,b; CNU–NX1–602a,b; CNU–NX1–610a,b.

Female external genitalia consist of a prominent ovipositor with two pairs of cutting valvulae (V1, V2), which extend backwards to the posterior edge of the 10th abdominal tergite, and markedly larger sheathing valvulae (V3) (Figs 7a and 8). Cutting valvulae (V1) are nearly straight with swollen bases that are connected to the basal plate of the ovipositor (bp) and the sternite of the 8th segment. Anterior part of bp is markedly concave between apophyses on the medial and lateral apodemes of the ovipositor basal plate (b,a) and its posterolateral part most probably represented by the anterior parts of the gonangulum (gon). Dorsal edge of V1 forming a longitudinal groove (aulax) and forms a sliding joint (olistheter), which enables the first and second pair of the valvulae to be moved in opposite directions, like the saw in the endophytic ovipositor of zygopteran Odonata[52]. The longitudinal ridge like keel (rhachis) on the ventral edge of V2 fit into the aulax (Fig. 8a,e). The surface of the distal part of V1 with 9–10 (11?) oblique prominent hook like ridges and approximately seven ridges on V2, probably function as a saw (Fig. 8f). The third pair of sheathing valvulae (V3) enclose the cutting valvulae when in a resting position. Sheathing valvulae are broader than the cutting valves and their surface bear scattered long setae (Fig. 8a,c). Surprisingly, the preservation is so good that it is possible to see in part the endoskeleton of V3, with two apophyses, aAp and pAp (see Fig. 7a). On the other hand, the detailed examination of the apical part of valvulae V3 of several specimens did not confirm the presence of a stylus as in *Permuralia* (Diaphanopterodea), *Monstropterum moravicum* (Paleodictyoptera) or modern Odonata[17,19,21]. The morphology of the ovipositor is unambiguously of endophytic type and in many aspects is comparable to that in modern damselflies (Odonata: Zygoptera), but sheathing valvulae (V3) clearly lack apical denticles in the form of a carina and an apical stylus[52]. Nevertheless, the experimental studies with extant endophytic Odonata shown that removal of styli has influenced position of eggs in egg sets which has to be considered as complex oviposition behavior driven by sensory organs on styli[53]. We assumed that such functionally sophisticated system of regular egg patterning in clutch evolved in some groups from less efficiently arranged oviposition. Surprisingly, the fossil record of endophytic oviposition can be traced back to the Pennsylvanian with the earliest evidence of scars as endophytic oviposition cavities on stem of *Calamites cistii* (Sphenophyta) known from Graissessac Basin in France reflecting rather irregular pattern of eggs in clutch[54]. While the fossil record of oviposition scars documented from younger strata (Permian) show generally more regular patterning of oviposition[55]. The recent discovery of endophytic oviposition in form of egg cavities arranged in longitudinal rows or zigzag configuration on leaf of *Cordaites* from the Pennsylvanian of Wettin member in Saale Basin strongly resambles the arrangement of eggs known in damselflies of Coenagrionidae (Zygoptera) and therefore the oviposition probably was caused by a member of the extinct odonatopteran suborder Archizygoptera[56]. So far the rich plant fossils from Xiaheyan locality given any evidence of oviposition scars yet and thus we cannot be sure to which plant *Brodioptera* layed its eggs.

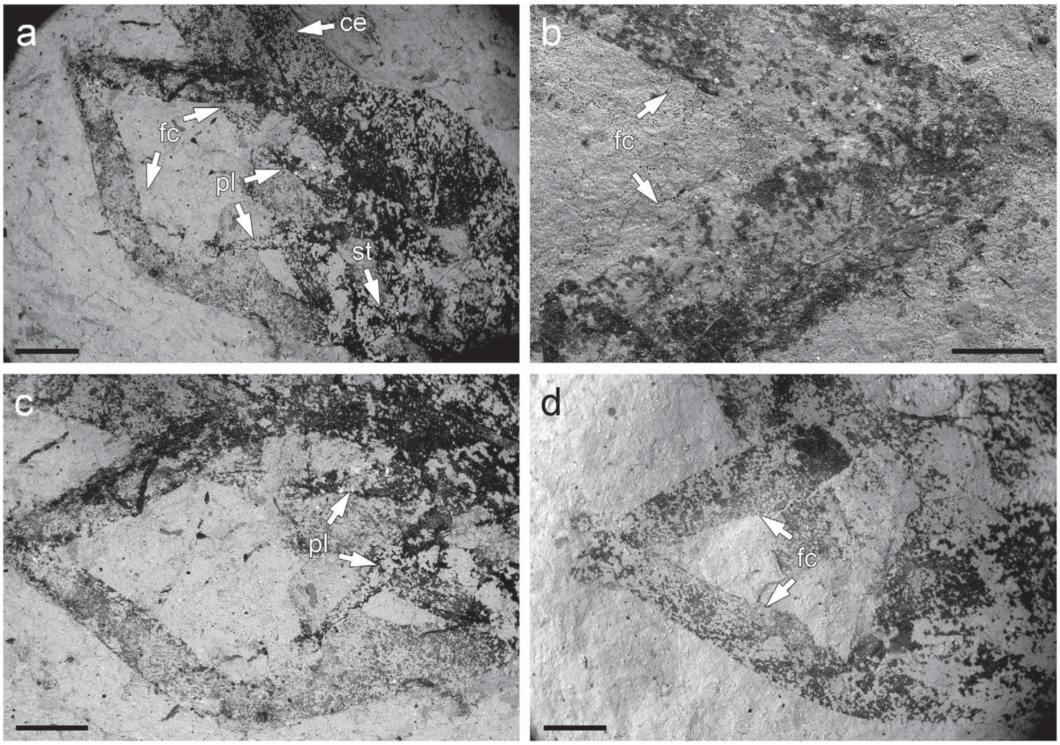

Figure 6. ***Brodioptera sinensis*, scanning electron micrographs of male external genitalia, Capital Normal University, Beijing, China**. (**a**) Forceps, penial lobes and styliger CNU–NX1–601a. (**b**) Detail of forceps apices with protruding setae CNU–NX1–601a. (**c**) Forceps and penial lobes CNU–NX1–601a. (**d**) Forceps CNU–NX1–610b. Arrows indicate cerci (ce), forceps (styli) (fc), penial lobes (pl) and styliger (st). Scale bars 600 μm (**a**), 200 μm (**b**), 500 μm (**c,d**).

Interestingly, the reduction of stylus on sheathing valvulae is also considered in case of stem odonatopteran *Erasipteroides valentini* (Erasipteridae) from Namurian of Hagen-Vorhalle in Germany, but its extreme length support rather endosubstratic oviposition[51]. On the other hand, the most primitive known fossil dragonflies Eugeropteridae had a short ovipositor.

Material examined: CNU–NX1–600a, b (holotype); CNU–NX1–613a; CNU–NX1–624a,b; CNU–NX1–651a,b.

Conclusions

For the first time, microstructures on the integumental surface were studied comprehensively on a large number of fossil specimens of a Megasecopteran species. In spite of the limitations imposed by the poor state preservation due the processes occurring during taphonomy this study revealed details of their microstructure and how selected body structures functioned in a Late Carboniferous insect, which lived approximately 317 Mya.

Our reconstruction of *B. sinensis* revealed it had a hypognathous head with prominent haustellate mouthparts in the form of a rostrum consisting of a basally short triangular labrum, two pairs of mandibulary and maxillary stylets, stout multi-segmented maxillary palps extending beyond the tip of stylets and covered with large sensory setae, and a labium with a pair of lobes underneath the stylets. We assume that these specialized piercing and sucking mouthparts were adapted for feeding on the spores of an unknown plant, like tree ferns or Cordaites. Antennae were filiform, reaching the tip of the stylets. Thorax with walking legs, narrow prothorax, meso- and metathorax approximately equal in size bearing two pairs of homonomous outstretched wings. The surface microstructure on the wings consists of irregularly scattered macrotrichia on the membrane and veins, which markedly decrease in density from the base to the apex of the wing, which possibly functioned as mechanoreceptors. Furthermore, it is likely that the fossils are of two different stages: teneral imago or subimago with hyaline wings with setae more clustered and a slender ovipositor always hidden in a third pair of valvulae and adults with dark coloured wing apices, less clustered setae on wings and ovipositor with the first two pairs of valvulae always more exposed than the third pair. Abdomen is 10-segmented and bears a pair of long multi-segmented cerci covered with protruding tactile sensory setae as in other members of the Palaeodictyopteroida. The most interesting feature are external copulatory organs that are rarely recorded in Carboniferous insects, which are present both sexes. Male genitalia consist of enlarged basal forceps (styliger), posteriorly slender two-segmented forceps curved distally and two basally enlarged penial lobes with slender apices for sperm transfer. We confirmed the presence of scattered long setae on the forceps and of a cluster of setae on the slender apices of the penial lobes. Female genitalia consist of an endophytic ovipositor with two pairs of cutting valvulae (V1 and V2) and a pair of enlarged sheathing valvulae (V3) covered with a scattering of long setae. Surfaces of the distal parts of V1 and V2

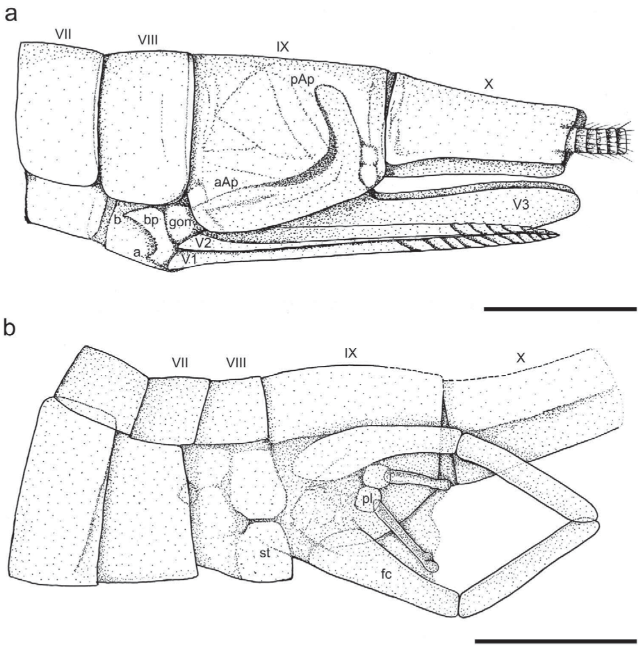

Figure 7. ***Brodioptera sinensis*, line drawings with partly reconstructed endoskeleton (drawn by MP).** (**a**) Distal part of abdomen bearing female external genitalia based on specimen CNU–NX1–651b. (**b**) Distal part of abdomen bearing male external genitalia based on paratype CNU–NX1–601a. Abbreviations: a/b – medial/lateral apodeme of basal plate of ovipositor, aAp/pAp – anterior/posterior apophysis of V3; au – aulax, bp – basal plate of ovipositor (lamina valvarum), ce – cerci, fc – forceps, gon – gonangulum, pl – penial lobes (penes), V1/V2/V3 – first/second/third valvulae of ovipositor, st – styliger (forceps base). Scale bars 3 mm (**a**), 2 mm (**b**).

with prominent hook like ridges are used for cutting plant tissue. Dorsal edge of V1 forming a longitudinal groove (aulax), which forms a sliding joint (olistheter) that enables the first and second pair of valvulae to move in opposite directions. The longitudinal ridge like keel (rhachis) on the ventral edge of V2 fits into the aulax as in modern endophytic zygopteran dragonflies[52]. Surprisingly, the third pair of valvulae lacks a stylus, which is well developed in few members of Diaphanopterodea and Palaeodictyoptera. Thus, in the Late Carboniferous this difference in the morphology of the ovipositor and associated behaviour was already established in the Palaeodictyopteroida. Nevertheless, a comparison of this trait in members of the Palaeodictyopteroida is not possible because there are only a few taxa with a third pair of valvulae.

Finally, it is likely that *B. sinensis* was a slow flying insect with a head bearing a long beak held in an hypognathous position, filiform antennae reaching the tip of the mouthparts and widely spread cerci as in recent mayflies (see Fig. 1). Nevertheless, flight in the dense hyperoxic Carboniferous atmosphere was easier in terms of the energy required[57].

Methods

Material and analysis. The material was a complete series attributed to *B. sinensis* consisting of 54 compressed fossils, including the holotype and paratype, ranging from fragmentary isolated wings to nearly complete specimens. Material was initially sorted mainly based on the presence of fine structures and quality of preservation. Twelve specimens were selected for environmental scanning electron microscopy and others were also examined for specific structures. All specimens examined in this study are housed in the Key Laboratory of Insect Evolution and Environmental Changes at Capital Normal University (prefix CNU-) in Beijing (China). Conventional study of the external morphology of all the available specimens using optical stereomicroscopy including the taxonomy was published[6]. Scanning electron micrographs of uncoated specimens were obtained using an environmental electron microscope Hitachi S-3700N (Hitachi Ltd, Chiyoda, Tokyo, Japan) at an accelerating voltage of 15 kV with a turntable sample holder at the Department of Paleontology, National Museum in Prague.

Terminology used for general insect morphology[58] and for external genitalia[59,60]. The terminology used for the description of the ultrastructure of setae[30,58]. The terms of macro- and microtrichia are classified based on their length (under and above 5 μm), based on presence vs. absence of setal socket and they are collectively referred as hairs. Large processes on wing are called bristles and tactile setae are called trichoid sensilla[30]. Naturally, without application of transmission electron microscopy for ultrastructure there is still some uncertainty to discern between these kinds of cuticular structures.

Abbreviations used for morphological structures are: ce – compound eyes, cl – clypeus, fl – flagelum, md – mandibulary stylets, mp – maxilary palps, mx – maxilary stylets, lb – labium, lm – labrum; abdomen: a/b– medial/lateral apodeme of basal plate of ovipositor, aAp/pAp – anterior/posterior apophysis of V3; au – aulax,

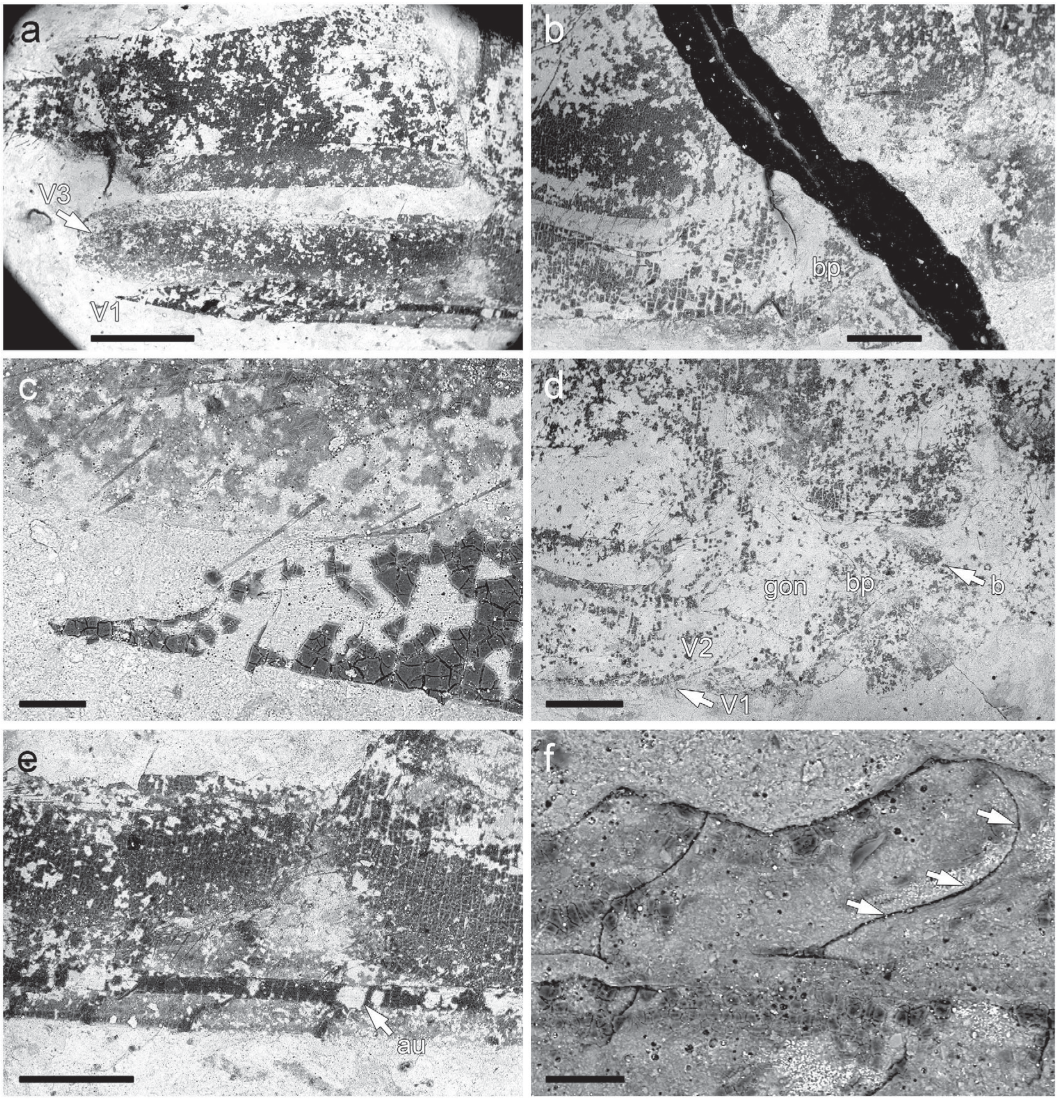

Figure 8. ***Brodioptera sinensis*****, scanning electron micrographs of female external genitalia, Capital Normal University, Beijing, China**. (**a–e**) Distal part of abdomen bearing female external genitalia CNU–NX1–651b. (**c**) Detail of ensheathing valvulae V3 bearing scathered setae. (**d**) Detail of ovipositor base with basal plate of ovipositor and gonangulum. (**e**) Detail of medial part of ovipositor with longitudinal groove (aulax) allowing a sliding joint (olistheter). (**f**) Detail of ovipositor valvulae V2 with prominent hook like ridges, CNU–NX1–624a. Scale bars 1000 μm (**a,b**), 100 μm (**c**), 500 μm (**d,e**), 50 μm (**f**).

bp – basal plate of ovipositor (lamina valvarum), ce – cerci, fc – forceps, gon – gonangulum, pl – penial lobes (penes), V1/V2/V3 – first/second/third valves of ovipositor, st – styliger (forceps base). The wing venation in general follows[61] and the nomenclature is adopted. Wing venation abbreviations: A1/A2 – first/second anal vein, CA/ CP – costa anterior/posterior, CuA/CuP – cubitus anterior/posterior, MA/MP – media anterior/posterior, RA/RP – radius anterior/posterior, ScP – subcosta posterior.

Outcrop location and age. All the insect specimens examined in this study came from Xiaheyan in Zhongwei County in the Ningxia Autonomous Region of northwestern China. Insects are preserved as compressed fossils in terrestrial facies of the Tupo Formation dated to Namurian B/C (early Bashkirian), which are biostratigraphically correlated with deposits in Europe, North America and Russia[62,63]. The paleoenvironment in which these sediments were deposited is interpreted as marine-lagoonal with tidal flats and marshlands[64].

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Author Contributions

J.P. and M.P. conceived the initial idea, D.R. provided fossil specimens to study, J.P. and M.P. examined material by light stereomicroscopy, performed ESEM analyses and prepared the figure plates, J.P., M.P. and D.R. wrote the paper.

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Článek 4

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General Palaeontology, Systematics and Evolution (Invertebrate Palaeontology)

Early Pennsylvanian aykhalids from Xiaheyan, northern China and their palaeogeographical significance (Insecta: Megasecoptera)

Les aykhalidés du Pennsylvanien inférieur de Xiaheyan, Nord de la Chine et leur signification paléogéographique (Insecta : Megasecoptera)

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ABSTRACT

The first members of the Palaeozoic insect family Aykhalidae (Megasecoptera) to be described were discovered in Siberia. Two new genera and three species from the Early Pennsylvanian (Namurian) Tupo Formation at Xiaheyan in northern China (Ningxia Hui Autonomous Region) are described on the basis of the patterns of their wing venation. An emended diagnosis of the Aykhalidae is provided based on the larger sample of specimens now available. *Sinopalaeopteryx* gen. nov. is established with two described species (*S. olivieri* sp. nov. and *S. splendens* sp. nov.). Monotypic *Namuroptera* gen. nov. is established for *N. minuta* sp. nov., a species that is markedly smaller in size, has a pointed connection between MA and RP, and very long brace m-cua well aligned with a short rp-m brace compared to *Aykhal* and *Sinopalaeopteryx*. All these newly described taxa extend our knowledge of the morphological disparity within the Aykhalidae. Due to the number of shared characters in wing venation, we consider Aykhalidae to be closely related to Sphecopteridae, with a broad distribution across Euramerica, Siberia and North China. In addition, this study documents individual and intraspecific variability in wing venation in the Aykhalidae and Sphecopteridae.

RÉSUMÉ

Les premiers représentants de la famille d'insectes paléozoïques Aykhalidae (Megasecoptera) décrits ont été découverts en Sibérie. Sur la base de leur nervation alaire, deux nouveaux genres et trois espèces sont décrits dans le Pennsylvanien inférieur (Namurien) de la formation Tupo dans la localité Xiaheyan, dans le Nord de la Chine (région autonome Ningxia Hui). Une diagnose amendée des Aykhalidae est proposée sur la base de ces nouvelles découvertes. *Sinopalaeopteryx* gen. nov. est établi avec deux espèces, *S. olivieri* sp. nov. et *S. splendens* sp. nov. Le genre monotypique *Namuroptera* gen. nov. est établi pour *N. minuta* sp. nov., une espèce de taille particulièrement petite. Ces taxons diffèrent nettement d'*Aykhal* et de *Sinopalaeopteryx* par leur plus petite taille, la connexion à angle aigu entre

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MA et RP, et une très longue nervure transverse m-cua bien alignée avec une courte nervure transverse rp-m. Toutes les espèces nouvellement décrites augmentent nos connaissances sur la disparité morphologique chez les Aykhalidae. En raison du nombre de caractères partagés dans la nervation alaire, nous considérons les Aykhalidae comme étroitement apparentés aux Sphecopteridae, famille à large distribution en Euramérique, Sibérie et Chine du Nord. En outre, notre étude nous permet de souligner la variabilité individuelle et intraspécifique de la nervation alaire chez les Aykhalidae et les Sphecopteridae.

1. Introduction

Palaeodictyopteroida Bechly, 1996 (= Palaeodictyopterida) has been conventionally considered to be a member of Palaeoptera, which includes four orders: Palaeodictyoptera, Megasecoptera, Permothemistida and Diaphanopterodea, the relationships among which are unresolved. Sroka et al. (2014) recently proposed Palaeodictyopterida as a sister group of Neoptera based on a cladistic analysis of palaeopteran groups including fossil and recent taxa, which was also previously suggested by Willmann (2004).

Rohdendorf (1962) proposed the concept Megasecopteroidea comprising Megasecoptera with wings permanently spread in the resting position, Diaphanopterodea with roof-like folded wings and Campylopterodea. The latter group was later rejected by Nel and Huguet (2002), who transferred the monotypic Campylopteridae to Odonataoptera. Several authors doubt the monophyly of Megasecoptera due to the lack of clear autapomorphy. Kukalová-Peck (1975) presented a list of characters for Megasecoptera and stated that each feature can occur also in Palaeodictyoptera. Riek (1976) proposed a key to the Megasecoptera with a division into two suborders, the Eumegasecoptera with the following characters "costal and subcostal spaces relatively broad, with stem of Cu diverging more or less regularly from wing base", and the Protohymenoptera with "costal and subcostal spaces very narrow, with Sc and R crowded close to costal margin and with basal sections of stems of main veins, with exception of anal vein, all crowded close to fore margin". Carpenter (1992) considered the Megasecoptera a palaeopterous order, which is likely to be transferred to the Palaeodictyoptera in the future because they share many characters. Sinitshenkova (2002: 120) established the order Mischopterida based on a fusion of Megasecoptera Brongniart, 1893 and Archodonata Martynov, 1932. Several families formerly included in the Megasecoptera were transferred to the order Dictyoneurida (= Palaeodictyoptera) and the status of suborders Eubleptina (~ Eubleptoidea Laurentiaux, 1953), Mischopterina (~ Eumegasecoptera), Aspidothoracina (~ Protohymenoptera), Permothemistina (~ order Permothemistida = Archodonata) were changed by the same author. Grimaldi and Engel (2005) proposed three lineages of Megasecoptera, with Eubleptoptera a sister group of Eumegasecoptera + Protohymenoptera mainly based on the different widths of their costal areas and other characteristics of their venation.

The present study deals with new members of Megasecoptera, which have wings that are usually markedly narrow at the base, frequently petiolate, generally reduced wing venation with branches on the main veins, narrow anal area, loss of archedictyon, few cross veins and long multisegmented cerci. The most obvious characteristic of megasecopteran wings is the form and shape of the anal area. There are two main types of anal area (Fig. 1). The first with two or more anal veins emerging from one point at the bases of the wings is present in Brodiopteridae and Xenopteridae (Fig. 1a) (Pinto, 1986; Pecharová et al., 2015). It is proposed that the latter family name be changed to Xenopteraidae (Ross et al., 2013). The second with one usually pectinate anal vein, which runs more or less parallel to the posterior wing margin, is present in the remaining families e.g., Sphecopteridae and Protohymenidae (Fig. 1b and c). Another frequently used character in the diagnosis of many families is the connection between veins RP-MA and M-CuA, which is rather variable in some families like the Brodiopteridae and Sphecopteridae, and very stable in others, e.g., Mischopteridae (see Fig. 1d–g).

The first published reference to the Namurian entomofauna in China is by Hong and Peng (1995). They presented a paper at the 18th Pacific Science Congress in Beijing. Hong (1998), proposed the name Qilianshan entomofauna for insects from Namurian C in the Qilianshan Mountains, and indicated they were the oldest entomofauna known from the North China palaeocontinent. Subsequent research resulted in several studies that focused on members of the following insect groups: Palaeodictyoptera, Odonatoptera, stem-Grylloblattodea, stem-Dictyoptera, stem-Orthoptera (Archaeorthoptera) and stem-Plecoptera (e.g., Béthoux et al., 2011; Li et al., 2013a; Liu et al., 2009; Prokop and Ren, 2007; Ren et al., 2008). Pecharová et al. (2015) briefly reviewed the previous studies on fossil insects and focused on describing the megasecopteran *Brodioptera sinensis* Pecharová, Ren and Prokop, 2015 based on an evaluation of more than 50 specimens. Such an extensive dataset for a single species is the source of our knowledge about intraspecific variability, which is rarely documented for Early Bashkirian insects.

The results presented here were partially previously elaborated during the preparation of the masters' thesis of the first author (Pecharová, 2013).

2. Material and methods

All specimens are deposited in the collection of Key Laboratory of Insect Evolution and Environmental Changes at Capital Normal University (prefix CNU-) in Beijing (China). Material consists of 14 compressed fossils mainly of fragments or complete wings. Due to the distortion of some

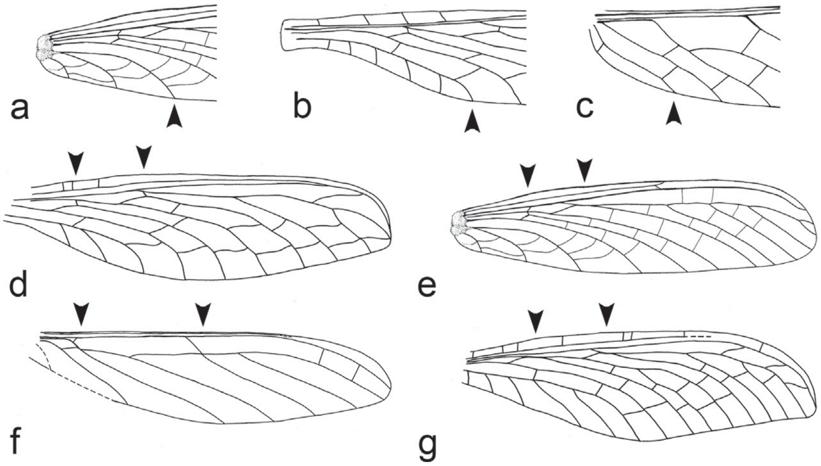

Fig. 1. Drawings of the wing venation of Megasecoptera with particular emphasis on main diagnostic characters (colour patterns omitted). **a**: *Brodioptera sinensis* Pecharová, Ren and Prokop, 2015 (Brodiopteridae); **b**: *Sphecoptera brongniarti* Meunier, 1908 (Sphecopteridae); **c**: *Permohymen schucherti* Tillyard, 1924 (Protohymenidae); **d**: *Foriria maculata* Meunier, 1908 (Foririidae); **e**: *B. sinensis* Pecharová, Ren and Prokop, 2015 (Brodiopteridae); **f**: *Scytohymen extremus* Martynov, 1937 (Scytohymenidae); **g**: *Corydaloides scudderi* Brongniart, 1885 (Corydaloididae). Abbreviations: **a–c**: arrangement of anal veins, A1 indicated by arrow; **d–g**: various types of connection between RP and MA (apical connection), M and CuA (basal connection) in the form of a brace or pointed fusion, positions indicated by arrows (drawings adopted or modified based on a personal re-examination by the authors).

Fig. 1. Dessins de nervations alaires de Megasecoptera, l'accent étant mis particulièrement sur les principaux caractères diagnostiques (les schémas en couleur ont été omis). **a** : *Brodioptera sinensis* Pecharová, Ren et Prokop, 2015 (Brodiopteridae) ; **b** : *Sphecoptera brongniarti* Meunier, 1908 (Sphecopteridae) ; **c** : *Permohymen schucherti* Tillyard, 1924 (Protohymenidae) ; **d** : *Foriria maculata* Meunier, 1908 (Foririidae) ; **e** : *B. sinensis* Pecharová, Ren et Prokop, 2015 (Brodiopteridae) ; **f** : *Scytohymen extremus* Martynov, 1937 (Scytohymenidae) ; **g** : *Corydaloides scudderi* Brongniart, 1885 (Corydalolididae) Abréviations : **a–c** : arrangement des veines anales, Al indiqué par des flèches ; **d–g** : types variés de connexion entre RP et AP (connexion apicale), M et CuA (connexion basale), sous forme d'une attache ou d'un point de fusion, positions indiquées par des flèches (dessins adoptés ou modifiés sur la base d'un réexamen par les auteurs).

of the fossils, we selected only seven for the description. The seven poorly preserved specimens (Nos. CNU-NX1-640, CNU-NX1-645a, b, CNU-NX1-648, CNU-NX1-668a, b, CNU-NX1-669a, b, CNU-NX1-670a, b, CNU-NX1-673) are considered therefore to have an uncertain systematic attribution. The main problem with these fossils is that it is difficult to interpret the basal part of the wing, which bears numerous diagnostic characters such as the connection of CuA with M. Therefore, we decided only to record these specimens because extensive field research at Xiaheyan is still ongoing and the discovery of additional material is anticipated.

Xiaheyan is situated in Zhongwei County in the Ningxia Hui Autonomous Region in northwestern China. The paralic development of the Tupo Formation with intercalated coal seams is biostratigraphically correlated with the Carboniferous strata in Europe, North America and Russia (Li et al., 1993, 2003; Wang, 2010). Zhang et al. (2013) provide the most comprehensive summary of the stratigraphy of the Tupo Formation correlated with Namurian B/C (Bashkirian) in Europe on the basis of occurrences of the following ammonoids (*Reticuloceras reticulatum*, *Gastrioceras listeri*, *G. montgomeryense*) and conodonts (*Declingnathodus noduliferous*, *Neognathodus symmetricus*). Insects are preserved as compressed fossils in greyish or black shales accompanied by plants, fish and bivalves (Zhang et al., 2013). Xie et al. (2004) indicate that the depositional environment was marine-lagoonal with tidal flats and marshlands.

The specimens were viewed using Leica MZ12.5 and Nikon SMZ 645 stereomicroscopes in a dry state and under a film of ethyl alcohol. Line drawings were made using a stereomicroscope with a camera lucida attachment. Photographs of fossils in a dry state or under a film of ethyl alcohol were taken using a Canon D550 digital camera equipped with MP-E 65 mm and EF 50 mm macro-lenses. Original photographs were processed using the image editing software Adobe Photoshop CS4 and some were also carefully processed using the stacking software Helicon Focus Pro.

The higher systematics of Megasecoptera is not satisfactorily resolved. The present work generally follows Riek (1976) and Carpenter (1992), and takes into consideration the comments of Sinitshenkova (2002) on this concept. Wing venation nomenclature generally follows the scheme of Kukalová-Peck (1991). Wing venation abbreviations: A1: first anal vein; CuA/CuP: cubitus anterior/posterior; MA/MP: media anterior/posterior; RA/RP: radius anterior/posterior; ScP: subcosta posterior; cross veins (braces) indicate veinal connections rp-ma, rp-m and m-cua.

3. Systematic palaeontology

Class INSECTA Linnaeus, 1758
Superorder PALAEODICTYOPTEROIDA Bechly, 1996
Order MEGASECOPTERA Brongniart, 1885
Suborder EUMEGASECOPTERA *sensu* Riek, 1976
Family AYKHALIDAE Sinitshenkova, 1993

Included genera. ***Aykhal*** Sinitshenkova, 1993 (type genus), *Sinopalaeopteryx* gen. nov., *Namuroptera* gen. nov.

Emended diagnosis. Wings elongate, petiole not obvious, costal area basally wide with several cross veins, widest part of costal area near wing base, ScP slightly longer than half the wing length and ending into RA before

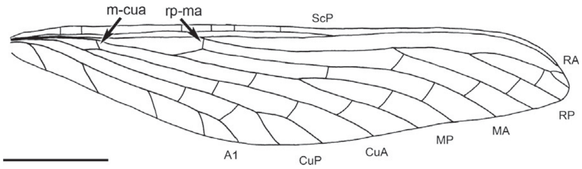

Fig. 2. *Sinopalaeopteryx splendens* gen. nov. et sp. nov., holotype CNU-NX1-641a (Capital Normal University, Beijing, China), line drawing of hindwing. Scale bar = 5 mm.

Fig. 2. *Sinopalaeopteryx splendens* gen. nov. et sp. nov., holotype CNU-NX1-641a (Capital Normal University, Pékin, Chine), dessin au trait d'aile arrière. Barre d'échelle = 5 mm.

first branching of RP, pterostigma slightly expressed or not obvious, RP multibranched, MA connected to RP at one point or by cross vein rp-ma (brace), MA simple, MP deeply forked, CuA simple, few simple cross veins arranged in a steplike pattern along posterior margin of the wing, single anal vein pectinate with at least four branches.

Genus ***Sinopalaeopteryx*** gen. nov.

Type species. *Sinopalaeopteryx splendens* sp. nov.

Etymology. Composite name after Sinae (China), palaeo (ancient) and pteryx (wing), masculine in gender.

Diagnosis. Based on wing venation. Wing basally narrow, broadest at about midwing, with stems of main veins basally close to each other, costal margin nearly straight, costal area widest at basal third gradually narrowing to midwing, ScP ending on RA proximal to the first branching of RP, RP with three branches, the first one simple or with terminal twig, MA connected by brace rp-ma with RP near the point where RA and RP divide, simple CuA connected by cross vein m-cua to M, first anal vein runs gradually parallel to the posterior wing margin.

Sinopalaeopteryx splendens sp. nov. (Figs. 2–4)

Etymology. Named in Latin after the brilliant state of its preservation.

Material. Holotype. CNU-NX1-641a, b (a) nearly a complete counter-imprint of a well-preserved insect wing; (b) rather fragmentary imprint of posterior part of the wing.

Supplementary material. CNU-NX1-667a, b, (isolated wing lacking apical third), CNU-NX1-672 (isolated wing lacking basal part).

Type stratum and locality. Tupo Formation, Carboniferous, Lower Pennsylvanian, Bashkirian, (equivalent to Namurian B–C), Xiaheyan Village, Zhongwei County, Ningxia Hui Autonomous Region, China.

Diagnosis. Wing of triangular shape, widest at about midwing, wing width markedly decrease from midwing to ending of MA, costal area with at least six perpendicular cross veins, ScP terminating on RA well before the first division of RP, CuP with terminal twig.

Description. Based on Holotype specimen No. CNU-NX1-641a, b.

Nearly complete wing of triangular shape, broadest at about midwing, membrane hyaline, several simple straight or oblique cross veins present between main longitudinal veins; costal margin raised basally forming a broad costal area with four perpendicular cross veins, concave ScP nearly straight ending on RA behind midwing, 15.5 mm from the wing base; RA + RP basally close to stem of M, separation of RA from RP about 1/3 along the wing length, RA simple reaching costal margin 1.1 mm from wing apex, RP pectinate ending in three main terminal branches that cover the apex of the wing, first branch with short terminal twig; division of MA and MP 1.4 mm basal of separation of RA and RP, simple convex MA diverging to RP with short connection by cross vein (brace) rp-ma just behind the separation of RA and RP; concave MP distally branched, ending in two branches on posterior wing margin; stem of Cu basally separated from RA + RP and M, division of CuA and CuP 4.5 mm from wing base, simple CuA diverging to M, shortly connected to M by cross vein (brace) m-cua; concave CuP with short terminal twig; anal area strongly reduced, single anal vein pectinate with 5 short branches.

Dimensions. *Holotype*. CNU-NX1-641a, b: Wing length 27.3 mm, maximum width 5.6 mm, distance from division CuA/CuP to first branch of RP 14.5 mm; CNU-NX1-667a, b: Preserved wing length 19.0 mm (estimated total wing length similar to the holotype), maximum width 5.4 mm, distance from division CuA/CuP to first branch of RP 14.2 mm; CNU-NX1-672: Length of preserved part of wing 17.7 mm (estimated total length 20.0 mm), maximum width 4.5 mm, distance from division CuA/CuP to first branch of RP 9.3 mm.

Discussion. *Sinopalaeopteryx* gen. nov. is attributed to the monotypic family Aykhalidae based on the following combination of wing venation characteristics:

- wing elongate without basal petiole;
- presence of rather broad costal area;
- ScP ending on RA slightly behind the midwing;
- RP multibranched;
- stems of M and Cu not fused at base of the wing;
- MP ends with two long branches (Sinitshenkova, 1993).

Aykhalidae were established for genus *Aykhal* with *A. helenae* Sinitshenkova, 1993 based on an isolated forewing from the Yakutia-Sakha deposits in Russia close to the boundary between Late Pennsylvanian and Early Permian (Zherikhin, 2002: 337). *Sinopalaeopteryx* gen. nov. differs from *Aykhal* in having a nearly straight costal margin (both fore- and hindwings), cross veins in costal area are sparsely arranged, brace rp-ma close to the point of division of RP and RA, brace m-cua is equal or longer than the part of CuA that emerges from CuP (in *Aykhal* this brace is shorter), first anal vein runs nearly parallel to the posterior wing margin. The lack of a pterostigma as in *Aykhal* could be considered as a variable character for Aykhalidae, due to its weak sclerotization in the holotype *A. helenae* Sinitshenkova, 1993, and therefore cannot be used as a significant character for separation (see Fig. 5). Due to the lack of prominent oblique cross veins between RA and RP distally and the aforementioned characters we propose separating *Sinopalaeopteryx* from the genus *Aykhal*. Moreover, we should noticed the Namurian age of *Sinopalaeopteryx* in contrast to the Gzhelian/Asselian age of *Aykhal*. Therefore, the designation of the genus *Sinopalaeopteryx* is well grounded and *Aykhal* is probably a closely related genus. Based on the triangular wing shape of *S. splendens* sp. nov. and its slightly reduced

Fig. 3. (Color online.) *Sinopalaeopteryx splendens* gen. nov. et sp. nov., holotype CNU-NX1-641a (Capital Normal University, Beijing, China), photograph of hindwing. Scale bar = 5 mm.

Fig. 3. (Couleur en ligne.) *Sinopalaeopteryx splendens* gen. nov. et sp. nov., holotype CNU-NX1-641a (Capital Normal University, Pékin, Chine), photographie d'aile arrière. Barre d'échelle = 5 mm.

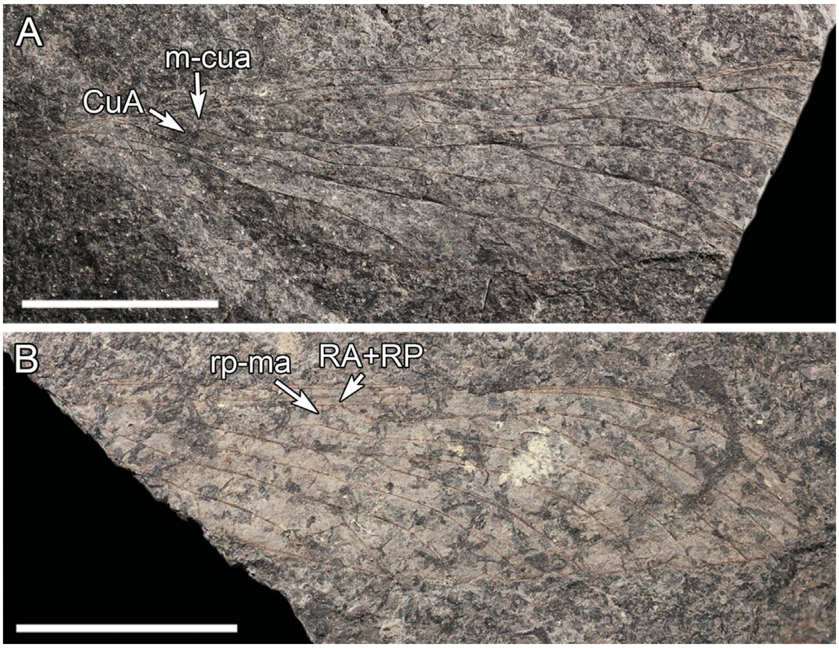

Fig. 4. (Color online.) *Sinopalaeopteryx splendens* gen. nov. et sp. nov. **A**. Photograph of specimen No. CNU-NX1-667a (Capital Normal University, Beijing, China). **B**. Photograph of specimen No. CNU-NX1-672 (Capital Normal University, Beijing, China). Scale bars = 5 mm.

Fig. 4. (Couleur en ligne.) *Sinopalaeopteryx splendens* gen. nov. et sp. Nov. **A**. Photographie du spécimen n° CNU-NX1-667a (Capital Normal University, Pékin, Chine). **B**. Photographie du spécimen n° CNU-NX1-672 (Capital Normal University, Pékin, Chine). Barre d'échelle = 5 mm.

costal area and costal margin without prominent serration, we consider that it is most probably a hindwing.

On the other hand, *Sinopalaeopteryx* gen. nov. shares a number of diagnostic characters with the genera *Sphecoptera* Brongniart, 1893 and *Cyclocelis* Brongniart, 1893, both placed in the family Sphecopteridae as they differ mainly in having petiolate wings (Carpenter, 1951: 345). However, petiolate wings in Megasecoptera is a rather

Fig. 5. (Color online.) *Aykhal helenae* Sinitshenkova, 1993. Photograph of holotype PIN No. 3838/1 (Palaeontological Institute of RAS coll., Moscow, Russia). Scale bar = 5 mm.

Fig. 5. (Couleur en ligne.) *Aykhal helenae* Sinitshenkova, 1993. Photographie de l'holotype PIN n° 3838/1 (Palaeontological Institute of RAS coll., Moscou, Russie). Barre d'échelle = 5 mm.

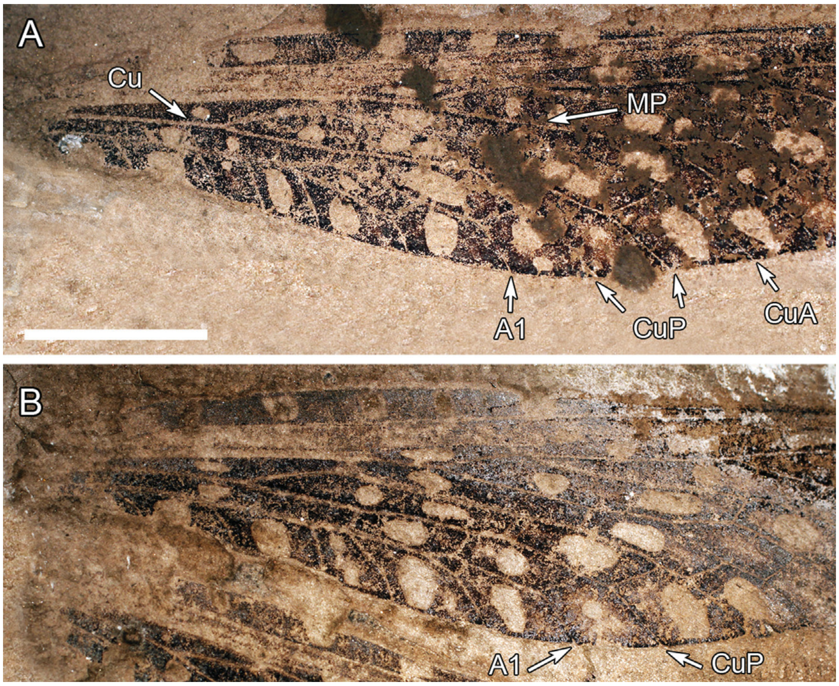

Fig. 6. (Color online.) *Cyclocelis chatini* Brongniart, 1893, syntype, No. R51136 (Muséum national d'histoire naturelle, Paris, France). **A**. Photograph of left forewing horizontally flipped over. **B**. Photograph of right forewing. Scale bar = 10 mm.

Fig. 6. (Couleur en ligne.) *Cyclocelis chatini* Brongniart, 1893, syntype, n° R51136 (Muséum national d'histoire naturelle, Paris, France). **A**. Photographie d'aile avant gauche, horizontalement basculée. **B**. Photographie d'aie avant droite. Barre d'échelle = 10 mm.

variable character and therefore difficult to use in systematics, e.g., family Bardohymenidae includes genera such as *Bardohymen* Zalessky, 1937 with clearly petiolate wings, but also other taxa that lack petioles, like *Calohymen* Carpenter, 1947. Sinitshenkova (2002: 121) has already recorded that the wings in both suborders of Mischopterida (Mischopterina and Aspidothoracina) of various taxonomic ranks are petiolate. Therefore, we consider this character to be variable as can be seen for instance in the Sphecopteridae, in which the wings of *Sphecoptera* spp. are generally more petiolate than those of *Cyclocelis* (Carpenter, 1951). Thus, in the future Aykhalidae is likely to be synonymized with Sphecopteridae due to the lack of significant diagnostic features with exception of that in all Sphecopteridae

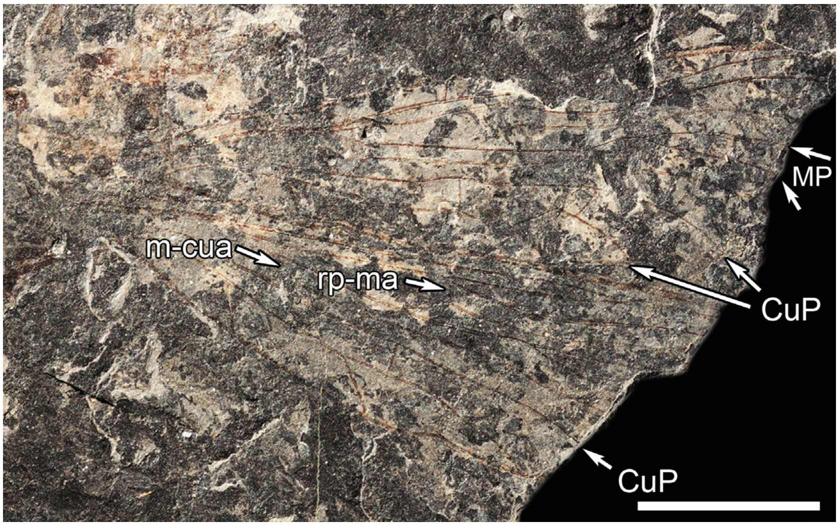

Fig. 7. (Color online.) *Sinopalaeopteryx olivieri* gen. nov. et sp. nov., specimen CNU-NX1-644b (Capital Normal University, Beijing, China), photograph of basal parts of fore- and hindwing with indication of branched CuP on forewing (horizontally flipped). Scale bar = 3 mm.

Fig. 7. (Couleur en ligne.) *Sinopalaeopteryx oliveri* gen. nov. et sp. nov., spécimen CNU-NX1-644b (Capital Normal University, Pékin, Chine), photographie des parties basales d'aile avant et arrière, avec indication de CuP branché sur l'aile avant (horizontalement basculé). Barre d'échelle = 3 mm.

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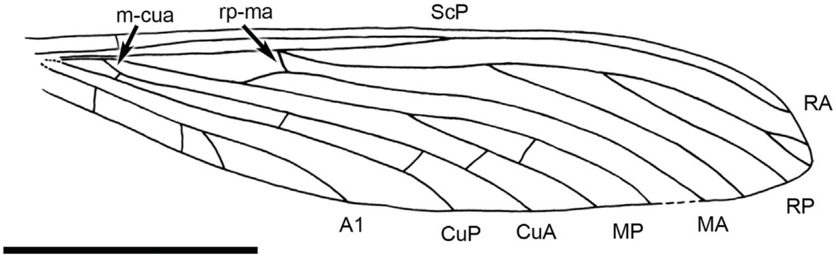

Figs. 8 and 9. (Color online.) *Sinopalaeopteryx olivieri* gen. nov. et sp. nov., holotype CNU-NX1-642a (Capital Normal University, Beijing, China), photograph and line drawing of wing. Scale bar = 5 mm.

Figs. 8. (Couleur en ligne.) *Sinopalaeopteryx olivieri* gen. nov. et sp. nov., holotype CNU-NX1-642a (Capital Normal Université, Pékin, Chine), photographie et dessin au trait d'aile. Barre d'échelle = 5 mm.

vein ScP ending well behind the first branching of RP, unlike in Aykhalidae where ScP ending before this point. Nevertheless, for consistency with current systematics we prefer to maintain Aykhalidae and Sphecopteridae as separate families until the discovery of more complete material resolves this problem.

Note. The family Sphecopteridae comprise two genera, *Sphecoptera* and *Cyclocelis* both known from Gzhelian of Commentry (France), a famous locality for exceptionally fine insect preservation. Supporting their possible relationship the genus *Cyclocelis* and *Sinopalaeopteryx* share a deeply branched MP vein and less petiolate wings among other characters. Nevertheless, re-examination of one of three syntypes of *Cyclocelis chatini* Brongniart, 1893 (all four wings preserved, No. R51136 housed in the Muséum national d'histoire naturelle, Paris) by one of us (MP) revealed variation in the venation in the basal part of the wing with CuP on the left forewing (and also right hindwing) either simple or with terminal twig. This difference is associated with a different arrangement of the first anal vein of the corresponding wing (see Fig. 6). Similar individual variability in CuP occurs in *Sinopalaeopteryx olivieri* sp. nov., where CuP has terminal twig only on one forewing (specimen CNU-NX1-644a, b) (Fig. 7). These examples of individual plasticity in some wing vein characteristics, which are commonly used in taxonomy, indicate that the careful selection of proper diagnostic characters is essential for stable systematics.

Sinopalaeopteryx olivieri sp. nov. (Figs. 7–10A)

Etymology. Named after the palaeoentomologist Dr. Olivier Béthoux who is also studying insect material from Xiaheyan.

Material. *Holotype*. CNU-NX1-642a, b (formerly 91NZ4033 and 91NZ1029): (a) imprint of four insect wings, one completely preserved, two lacking basal parts and one folded and deformed in medial part (b) counter-imprint poorly preserved.

Supplementary material. CNU-NX1-644a, b (fragmentary preserved head, two pairs of wings incompletely preserved, abdomen with sclerotized ovipositor ventrally preserved, cerci). CNU-NX1-647a, b: (a) partially preserved head with short incomplete mouthparts, two nearly completely preserved wings, two fragmentary preserved wings, fragmentary preserved male genitalia, (b) apical parts of two wings.

Type stratum and locality. Tupo Formation, Carboniferous, Lower Pennsylvanian, Bashkirian, (equivalent to Namurian B–C), Xiaheyan, Zhongwei County, Ningxia Hui Autonomous Region, China.

Diagnosis. Wings homonomous, wing widest at about midwing, posterior margin evenly curved, from midwing to ending of MA nearly straight, ScP terminating on RA near the level of the first branching of RP, CuP simple or with terminal twig, brace m-cua almost three times longer than the short oblique CuA emerging from CuP.

Description. Based on Holotype specimen No. CNU-NX1-642a, b.

Both pairs of homonomous wings basally slender, membrane probably originally hyaline without a colour pattern; length of wing about 17.0 mm, width 3.6 mm at widest part; irregular cross veins sparsely distributed along posterior part of wing; anterior margin of wing slightly basally convex forming costal area with at least one perpendicular cross vein; ScP slightly bowed and terminating on RA beyond midwing, about 6.9 mm from wing apex; division RA and RP at about 1/3 of the wing length; simple RA

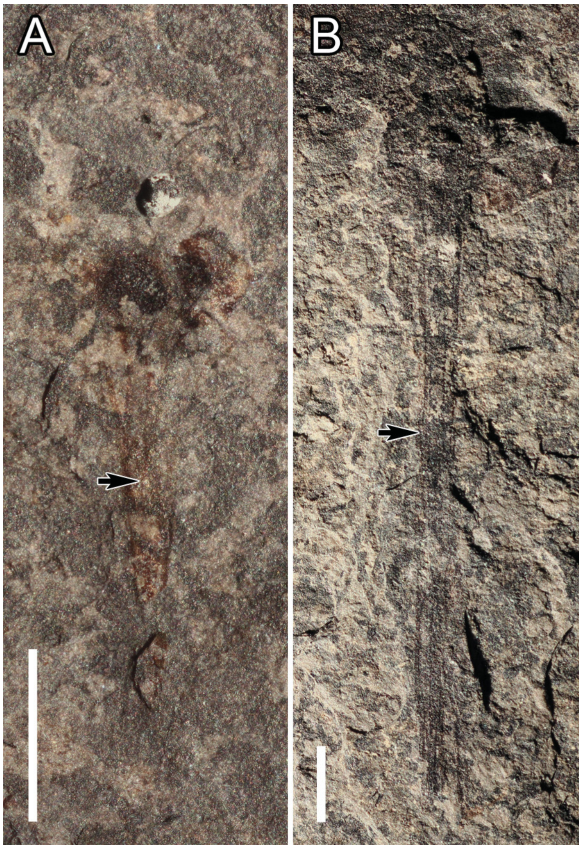

Fig. 10. (Color online.) Two types of rostrum-like mouthparts from Xiaheyan (China). **A**. *Sinopalaeopteryx olivieri* gen. nov. et sp. nov. (Aykhalidae), specimen CNU-NX1-647a (Capital Normal University, Beijing, China), detailed photograph of head with stout rostrum. **B**. *Brodioptera sinensis* Pecharová, Ren and Prokop, 2015, specimen CNU-NX1-609b (Capital Normal University, Beijing, China), detailed photograph of head with slender elongate rostrum, arrows indicate rostrum. Scale bars = 1 mm.

Fig. 10. (Couleur en ligne.) Deux types de parties de la bouche de type rostre en provenance de Xiaheyan (Chine). **A**. *Sinopalaeopteryx olivieri* gen. nov. et sp. nov. (Aykhalidae), spécimen CNU-NX1-647a (Capital Normal University, Pékin, Chine), photographie de détail de la tête avec un rostre robuste. **B**. *Brodioptera sinensis* Pecharová, Ren et Prokop, 2015, spécimen CNU-NX1-609b (Capital Normal University, Pékin, Chine), photographie de détail de la tête avec un rostre allongé mince ; les flèches indiquent le rostre. Barre d'échelle = 1 mm.

running parallel to anterior margin of wing reaches it close to apex; RP pectinate with three main branches, first branch simple or with terminal twig; M basally adjacent to radius, division of MA and MP based of separation of RA and RP; convex MA simple, basally connected by a short cross vein (brace) to division point of RA + RP; concave MP distally branched ending with two branches on posterior margin of wing; stem of Cu basally separated from RA + RP and M, division of CuA and CuP 3.2 mm from wing base, simple CuA diverging to M, shortly connected to M by cross vein (brace); simple CuP; anal area formed by single pectinate anal vein with four branches.

Dimensions. Holotype CNU-NX1-642a, b: Wing length 17.0 mm, maximum width 3.5 mm, CNU-NX1-644a, b: Length of best-preserved wing 16.5 mm, maximum width 3.4 mm, CNU-NX1-647a, b: Length of head including mouthparts 2.6 mm, forewing length 18.0 mm, maximum width 4.9 mm, hindwing length 18.6 mm, maximum width 4.9 mm.

Discussion. This fossil clearly belongs to the genus *Sinopalaeopteryx* as it has all of the diagnostic characters. Holotype of *S. olivieri* sp. nov. has four wings in various states of preservation, but it is impossible to distinguish between fore- and hindwings. Wings are homonomous without any sign of triangular shape as occurs in *S. splendens* sp. nov. Several differences in wing venation can be used to separate *S. olivieri* sp. nov. from *S. splendens* sp. nov.:

- ScP ends closer to the point of separation of the first branch of RP;
- brace m-cua distinctly much longer;
- wings markedly smaller in comparison to the latter species.

Cross veins are rather sparse in *S. olivieri* and numerous in *S. splendens*. However, this can be due to poor preservation.

The most apical branch of RP in *S. olivieri* is either simple or with short terminal twig. This example indicates possible problems associated with using "short terminal twig" as a diagnostic character in taxonomy.

Partly preserved head with mouthparts in form of stylets (No. CNU-NX1-647a) is discussed below (Chapter 4).

Genus ***Namuroptera*** gen. nov.

Type species. *Namuroptera minuta* sp. nov. here designated.

Etymology. Composite name after Namurian age and -ptera, feminine in gender.

Diagnosis. Based on wing venation; costal area with at least three perpendicular cross veins, ScP ending on RA beyond the midwing, near the level of the first branching of RP; MA connected at one point to RP; RP with three branches, the first one simple or with terminal twig, m-cua brace longer than a short oblique part of CuA emerging from CuP, aligned with a short brace rp-m; CuP simple; the first anal vein runs gradually parallel to posterior wing margin.

Namuroptera minuta sp. nov. (Figs. 11 and 12)

Etymology. Name based on its small size, in Latin.

Material. *Holotype*. CNU-NX1-646a, b (male), (a) counter-imprint of insect with partly preserved head with antenna, both pairs of wings with distinct wing venation markedly deformed, distal part of abdomen with gonopods and incomplete cerci; (b) imprint of fragmented head and a well-preserved set of wings.

Type stratum and locality. Tupo Formation, Carboniferous, Lower Pennsylvanian, Bashkirian, (equivalent to Namurian B–C), Xiaheyan, Zhongwei County, Ningxia Hui Autonomous Region, China.

Description. Based on Holotype specimen No. CNU-NX1-646a, b (male).

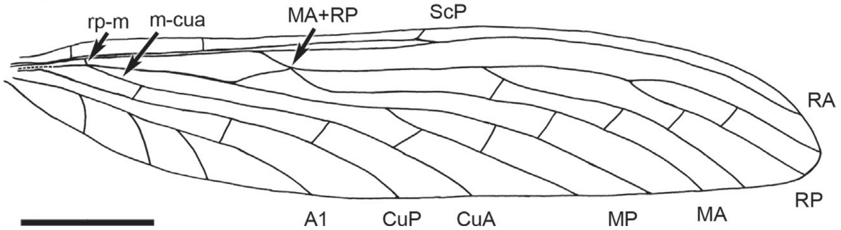

Fig. 11. *Namuroptera minuta* gen. nov. et sp. nov., holotype CNU-NX1-646b (Capital Normal University, Beijing, China), line drawing of the best preserved wing. Scale bar = 2 mm.

Fig. 11. *Namuroptera minuta* gen. nov. et sp. nov. holotype CNU-NX1-646b (Capital Normal University, Pékin, Chine), dessin au trait de l'aile la mieux préservée. Barre d'échelle = 2 mm.

Caput. Poorly preserved with possible internal structures (endoskeleton) and multisegmented long antennae.

Thorax. Fragmentarily preserved, prothorax markedly elongated.

Wings. Both pairs basally broad, membrane hyaline, forewing and hindwing nearly homonomous with broader costal area in forewings; venation slightly distorted (corresponding measurements given for right hindwing); costal margin slightly convex; ScP reaching RA well beyond midwing, 5.8 mm from wing apex, short cross vein present between ScP and anterior wing margin 0.2 mm before tip of ScP; costal area basally broad with several oblique or perpendicular cross veins present in basal half of wing area; radius slightly basally bowed, separation of RA from RP in basal third of the wing, 4.2 mm from wing base, RA reaching anterior wing margin well before wing apex, RP pectinate ending in three branches that cover wing apex, first branch simple or with terminal twig; stem of M running basally close to radius, division of MA and MP 3.8 mm from the wing base, convex simple MA strongly diverging to RP and connected at one point; concave MP ending with two long branches on posterior wing margin, division of CuA and CuP 2.1 mm from wing base, convex simple CuA connected with M by very long brace m-cua (0.8 mm long) aligned with short brace rp-m; concave CuP simple, single anal vein pectinate reaching posterior wing margin with four simple branches.

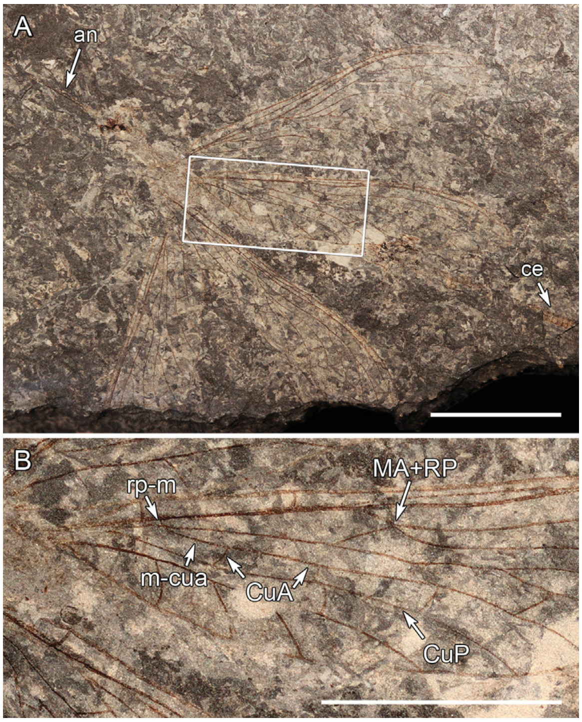

Fig. 12. (Color online.) *Namuroptera minuta* gen. nov. et sp. nov., holotype CNU-NX1-646b (Capital Normal University, Beijing, China). **A**. Photograph of habitus with marked part in detail, [an: antenna; ce: cerci]. **B**. Detail of basal part of hindwing. Scale bars = 5 mm (A), 3 mm (B).

Fig. 12. (Couleur en ligne.) *Namuroptera minutra* gen. nov. et sp. nov., holotype CNU-NX1-646b (Capital Normal University, Pékin, Chine). **A**. Photographie de l'habitus avec la partie encadrée pour le détail [an : antenne ; ce : cerci]. **B**. Détail de partie basale d'aile arrière. Barres d'échelle = 5 mm (A), 3 mm (B).

Abdomen. Male gonopods present, the terminal abdominal segment with a pair of segmented stout cerci bearing dense setation.

Dimensions. Holotype CNU-NX1-646a, b: estimated body length 13.5 mm, wing length 12.5 mm (the most complete without visible distortion), maximum width 2.5 mm.

Discussion. First, this fossil has the same pattern of venation as the family Sphecopteridae but differs mainly in having basally broader wings and an RA that does not reach the apex of the wing. The combination of the following characters:

- elongate nearly homonomous wings not petiolate basally;
- costal area basally broad with several cross veins;
- ScP ending on RA beyond midwing;
- RP with several branches;
- stems M and Cu basally well separated;
- MP branched,

support the placement of this fossil in the Aykhalidae (Sinitshenkova, 1993).

Namuroptera gen. nov. differs in the presence of a pointed connection between MA and RP and very long brace m-cua and well aligned short brace rp-m, unlike in *Aykhal* and *Sinopalaeopteryx*. In addition, *Namuroptera* markedly differs in the smaller size of its wings, which are about 12 mm long compared to a range of 17 to 27 mm for *Aykhal* and *Sinopalaeopteryx*. Nevertheless, *Namuroptera* provides evidence for a disparity in the pattern of venation in Aykhalidae.

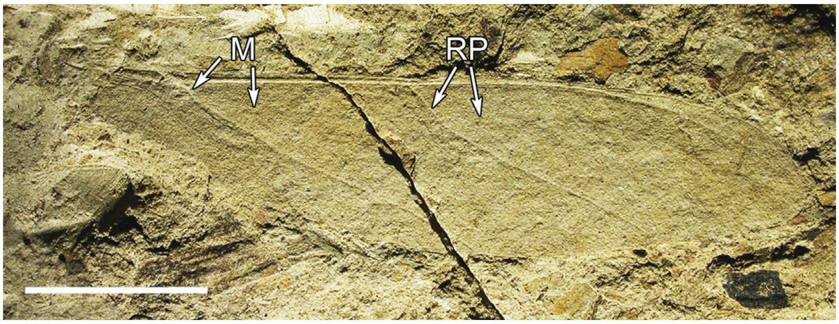

Fig. 13. *Scytohymen extremus* Martynov, 1937, holotype No. 2/99 (Palaeontological Institute of RAS coll., Moscow, Russia), photograph of wing. Scale bar = 10 mm.

Fig. 13. *Scytohymen extremus* Martynov, 1937, holotype n° 2/99 (Palaeontological Institute of RAS coll., Moscou, Russie), photographie d'aile. Barre d'échelle = 10 mm.

In particular, the course of vein CuA beyond its separation from CuP, markedly changes at the level of brace m-cua from strongly convex (proximal part) to neutral or concave (distal part). We suspect this is similar to the course of vein RP at the point of contact with MA as can be seen in *Scytohymen extremus* Martynov, 1937 (Fig. 13).

Note. The fossils from Ningxia are often badly distorted with hardly identifiable veinal splits. This can be seen on the right hindwing of CNU-NX1-646a, b (Fig. 12), where vein RA seems to run over ScP, which is not the case on the other wings. This is a result of postmortal distortion.

4. Morphology of the mouthparts of Aykhalidae

The head and mouthparts of Aykhalidae are only preserved in *S. olivieri* (suppl. spec. No. CNU-NX1-647a), which clearly show rather short and basally stout stylets and fragmentarily preserved other parts, possibly the eyes (see Fig. 10A). However, the lengths of the stylets are half the length of those of *B. sinensis* (Brodiopteridae), in which the stylets are half the length of the body (see Fig. 10B) (Pecharová et al., 2015). The occurrence of both types at the same locality suggests probable adaptation to feeding on a different plant, if these insects are herbivorous. Variation in size of stylets among Palaeodictyopteroida is rather broad and associated with their specialization for feeding on particular plants or tissues of plants (Shear and Kukalová-Peck, 1990). Labandeira and Phillips (1996) described palaeodictyopteran piercing-and-sucking damage to the phloem and xylem of the marattialean tree fern *Psaronius chasei* from the Late Pensylvanian in the Illinois Basin. Shcherbakov et al. (2009) report Cordaite seeds with punctures made by palaeodictyopteroids (?Megasecoptera) in the Early Permian of Russky Island, South Primorye in Russian Far East. So far, the palaeobotanical record for Xiaheyan has revealed a fascinating fructification of *Nudasporestrobus ningxicus* with *in situ* megaspores possibly attributed to a lycopsid *Sigillaria* sp. The large cylindrical strobilus has an incomplete peduncle with sporophylls arranged in ascending spirals, each bearing a sporangium with megaspores *in situ* (Feng et al., 2008). Such plants are a rich food source for sucking insects, but so far, there is no evidence of piercing or sucking damage to these plants.

5. Conclusions

Early Namurian insects are currently the oldest unambiguous records of Pterygota or winged insects, a novelty undoubtedly responsible for their success (Engel et al., 2013; Nicholson et al., 2014). Megasecopterans are among the groups with members already known since the Namurian B from deposits in Europe and North America (Brauckmann et al., 2003; Nelson and Tidwell, 1987). The recent discovery of the Chinese locality at Xiaheyan revealed an unexpected diversity of insects. The presence of *B. sinensis* Pecharová, Ren and Prokop, 2015 (Megasecoptera: Brodiopteridae) supports relationships with species known from Europe [*B. pintoi* (Brauckmann et al., 2003)] and North America (*B. cumberlandensis* Copeland, 1957 and *B. stricklani* Nelson and Tidwell, 1987) of a similar age (Pecharová et al., 2015). Moreover, there are many megasecopterans along with the odonatopterans, archaeorthopterans and grylloblattodeans discovered at this locality. At least, it seems that they are the most abundant group of palaeodictyopteroids after the scarce Palaeodictyoptera, for which there are two described species (Li et al., 2013b; Prokop and Ren, 2007).

Two new genera and three species of megasecopteran Aykhalidae, *S. olivieri* gen. nov., sp. nov., *S. splendens* sp. nov. and *Namuroptera minuta* gen. nov., sp. nov. from Xiaheyan, together with known *A. helenae* from Gzhelian/Asselian of Yakutia-Sakha deposits in Siberia reveal unique links with the family Sphecopteridae known from the Gzhelian in Europe (Commentry, France). This close relationship is based on a number of shared characteristics of wing venation, which is also supported by the time span and broad distribution. This discovery also reveals that pattern in wing venation of some related megasecopteran families remained stable during the Pennsylvanian.

In addition, we document intraspecific (and individual) variability in the veins RP and CuP of members of the family Aykhalidae (*S. olivieri* gen. nov., sp. nov.) and Sphecopteridae (*C. chatini* Brongniart, 1893). Finally, the polarity of the course of vein CuA in *N. minuta* gen. nov., sp. nov. beyond its separation from CuP changes markedly at the level of brace m-cua from strongly convex to neutral or concave. We suspect this is comparable to the course of vein RP at the point of contact with MA as can be seen in *S. extremus* Martynov, 1937.

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Článek 5

Prokop, J., Pecharová, M., Nel, A., Grey, M. & Hörnschemeyer, T. 2017. A remarkable insect from the Pennsylvanian of the Joggins Formation in Nova Scotia, Canada: insights into unusual venation of Brodiidae and nymphs of Megasecoptera. *Journal of Systematic Palaeontology*;
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Podíl autorů: JP: korespondenční autor, texty, studium materiálu, fotografie; **MP**: kresby, část popisů, část fotografií, interpretace křídelní žilnatiny; **AN**: úprava textů; **MG**: poskytnutí materiálu, informace o lokalitě, jazyková úprava; **TH:** úprava textů.

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A remarkable insect from the Pennsylvanian of the Joggins Formation in Nova Scotia, Canada: insights into unusual venation of Brodiidae and nymphs of Megasecoptera

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The discovery of a new megasecopteron, *Brodia jogginsensis* sp. nov., is reported from the Joggins Formation, a UNESCO World Heritage Site in Nova Scotia, Canada. The new species diagnosis is based on the unique pattern of hind wing venation bearing three anterior branches of MP and pectinate CuP with four posterior branches. Its occurrence in Langsettian deposits of the Maritimes Basin correspond well with other Pennsylvanian Brodiidae known from the Coal Measures of the UK and Mazon Creek in Illinois, USA. Based on our re-examination, we propose to transfer the genus *Pyebrodia*, with *P. martinsnetoi* Brauckmann & Herd, 2003, described as a putative member of Brodiidae, into Palaeodictyoptera family *incertae sedis* as it lacks most of the diagnostic features of Brodiidae. The holotype of *Eubrodia dabasinskasi* Carpenter, 1967, housed in a private collection, was supplemented by the description of an additional specimen housed in The Field Museum, Chicago. The fossil record of Brodiidae is reviewed and additional remarks concerning the wing morphology and the function of specialized structures, such as reinforcements in the form of a specialized crossvein between A1 and CuP, are given. Putative nymphs of Brodiidae and development of wings in Megasecoptera are discussed.

http://zoobank.org/urn:lsid:zoobank.org:pub:741846CB-FAD0-4103-90D3-2896D8909E94

Keywords: Insecta; Palaeodictyopterida; wing venation; postembryonic development; Bashkirian; Maritimes Basin

Introduction

Megasecoptera are specialized extinct Palaeodictyopterida bearing outstretched homonomous and slender wings with markedly corrugated longitudinal veins and mouthparts in the form of a rostrum with elongate stylets. Brodiidae are one of the families with markedly stalked wings, allowing slow, near-hovering flight, functionally resembling the wings of extant damselflies (Odonata: Zygoptera) (Wootton & Kukalová-Peck 2000). The presence of a dense archedictyon in the wing venation of some members has made certain authors doubt the placement of Brodiidae within Megasecoptera (Handlirsch 1906; Bolton 1921). The group is currently known from early to middle Pennsylvanian Coal Measure deposit of Coseley, Sedgeley and Dudley, Staffordshire in the UK, Mazon Creek in Illinois, USA, and Piesberg, Lower Saxony, Germany (Carpenter 1992; Brauckmann & Herd 2003). However, the fossil record of Brodiidae is mainly based on isolated wings or parts thereof. A single specimen of *Eubrodia dabasinskasi* Carpenter, 1967, in the private collection of Helen Piecko (HTP 433), also preserves parts of other body structures (Carpenter & Richardson 1971, figs 12, 13). In addition, incompletely developed curved wing pads of supposed immature stages found in Coseley were attributed to *Brodia priscotincta* Scudder, 1881 by Bolton (1921). Handlirsch (1911, p. 375) described an immature megasecopteran from a Mazon Creek nodule as *Lameereites curvipennis*, the wing tracheation strongly resembling the specimens described by Bolton. Carpenter (1967) pointed out that *Lameereites* probably belongs to Brodiidae based on the pattern of wing pad tracheation, but there is no certainty in this assignment.

The Maritimes Basin and the Cumberland sub-basin are two sequences well-known for historical records of Carboniferous insects, including the famous 'Fern Ledges' locality (New Brunswick, Canada) known since the mid-nineteenth century and the Joggins coalfield locality (Nova Scotia, Canada) (e.g. Scudder 1865; Dawson 1867). Copeland (1957) described from the Joggins

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*Corresponding author. Email: jprokop@natur.cuni.cz coalfield the first Brodiopteridae whose relatives, such as *Brodioptera stricklani* Nelson & Tidwell, 1987 from the Namurian of Utah, currently represent the oldest Megasecoptera and are therefore among the earliest recorded Pterygota (see Brauckmann *et al.* 1996; Prokop *et al.* 2005; Engel *et al.* 2013; Pecharová *et al.* 2015a).

The material studied here comes from the Joggins Fossil Cliffs (Nova Scotia, Canada), a UNESCO World Heritage Site for the Late Carboniferous (Pennsylvanian). The fossil biota from the Joggins Formation within this site indicates that three primary ecosystems existed through 14 cycles of brackish bays, terrestrial wetlands, and terrestrial drylands (Falcon-Lang *et al.* 2006). Nevertheless, insect remains are extremely scarce in this formation, with only the specimen described here and a small fragment of a wing apex (NSM 0096F) attributed tentatively to the palaeodictyopteran family Spilapteridae.

This study describes a new species of *Brodia* from the Joggins Formation showing for the first time brodiids with fore- and hind wings (although fragmentarily preserved) and provides a review of our current knowledge of this megasecopteran family. Remarks are also given on the morphology and development of wings in Brodiidae and other Megasecoptera.

Material and methods

A single specimen (NSM008GF031.092) preserved in a large slab of grey mudstone is stored in the collection of the Joggins Fossil Institute, Joggins, Nova Scotia, Canada. This holotype of *Brodia jogginsensis* sp. nov. was observed using a Nikon SM-Z stereomicroscope in a dry state and under a thin film of ethyl alcohol. The preservation of the insect specimen on the large slab required the line drawing restoration of the wing venation to be based on several photographs with variously oriented spot lighting and application of a Wacom Intuos drawing tablet (highlighted contours) with additional corrections based on observational notes. Photographs were taken with a Canon D550 digital camera with attached macro lenses EF 50 mm and MP-E 65mm for fine-scale details, and with a Nikon Coolpix S8000 for habitus. The original photographs were processed using Adobe Photoshop CS6 and, for some images, the focus-stacking software Helicon Focus Pro 6.2.2 was used.

The taxonomy of Brodiidae follows Carpenter (1967, 1992). The wing venation conception in general follows Lameere (1922, 1923) and nomenclature for the veins is adopted from Kukalová-Peck (1991).

Institutional abbreviations

Specimens from the following institutional collections were also examined for comparative purposes: **BU**: Lapworth Museum of Zoology, University of Birmingham, Birmingham, UK; **FMNH**: The Field Museum of Natural History, Chicago, IL, USA; **MNHN**: Muséum national d'Histoire naturelle, Paris, France; **MCZ**: Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; **MSO**: Museum am Schölerberg, Osnabrück, Germany; **NHMUK**: Natural History Museum, London, UK; **ROM**: Royal Ontario Museum, Toronto, Canada; **YPM**: Yale Peabody Museum, New Haven, CT, USA.

Anatomical abbreviations

The following symbols are used for the wing veins or tracheae (symbols in capitals denote the longitudinal veins/tracheae): **CA**/**CP**, costal anterior/posterior; **ScA**/**ScP**, subcostal anterior/posterior; **RA**/**RP**, radial anterior/posterior; **MA**/**MP**, medial anterior/posterior; **CuA**/**CuP**, cubital anterior/posterior; **A1**, first anal vein; **cup-a**, specialized crossvein.

Other abbreviations used for the morphology of head structures are: **an**, antennae; **ce**, compound eyes; **lb**, labium; **md**, mandibular stylets; **mx**, maxillar stylets.

Systematic palaeontology

Order **Megasecoptera** Brongniart, 1885
Family **Brodiidae** Handlirsch, 1906

Emended diagnosis. Wings petiolate, costal margin bearing prominent irregular tubercles; short convex ScA diverges obliquely towards costal margin; ScP nearly straight, costal area broad basally; RA simple, ending in wing apex, subcostal area broad in distal part; RP diverges from RA approximately in the basal third of wing, ending pectinate with three to five posterior branches; M basally separated from RA+RP; MA diverges from MP directed towards RP; convex MA reaching posterior margin simple; MP pectinate ending with at least two terminal branches; CuA simple; CuP posteriorly pectinate ending with at least two branches; anal area narrow strongly reduced; specialized crossvein connecting veins CuP and A1; posterior wing margin with small spines particularly in basal part.

Included genera. *Brodia* Scudder, 1881 (type genus), *Eubrodia* Carpenter, 1967.

Genus ***Brodia*** Scudder, 1881

Type species. *Brodia priscotincta* Scudder, 1881.

Brodia jogginsensis sp. nov.
(Figs 1, 2)

2006 Megasecoptera, family indet. Falcon-Lang *et al.*: 566.

Diagnosis (based on hind wing venation). Wing elongated, MP pectinate with three anterior branches, CuA and CuP running closely parallel toward posterior wing margin, CuP posteriorly pectinate ending with four branches, simple straight or oblique crossveins between the main branches MP and CuA.

Etymology. Named after the Joggins Formation where holotype specimen was found.

Holotype. NSM008GF031.092 (positive of fragmentary thorax with short prothorax, meso- and metathorax of sub-circular shape, two pairs of outstretched elongated wings, 3 (?4) basal abdominal segments preserved in grey mudstone); stored in the Joggins Fossil Institute, Joggins, Nova Scotia, Canada.

Type locality. The sedimentary rocks of the Cumberland Group, Pennsylvanian, contain five formations that include the Joggins Formation (described below); this group is part of the UNESCO World Heritage Site.

Type stratum. Joggins Formation, Early Pennsylvanian, mid-Bashkirian, Langsettian, after palynological analyses of Utting *et al.* (2010), with a probable age of *c.* 313.4–314.5 Ma (Falcon-Lang *et al.* 2006).

Description. Meso- and metathorax of sub-circular shape, rather poorly preserved. Both pairs of nearly homonomous elongate wings in outstretched position, wings shortly petiolate with narrow bases, markedly reduced anal area, wing span about 114 mm. Forewings slightly longer than hind wings, both widest at about mid wing; ScP (preserved partially on right forewing only) strongly concave, running parallel to radius, separating basally rather broad costal area narrowing distally; RA strongly convex running towards wing apex, concave or

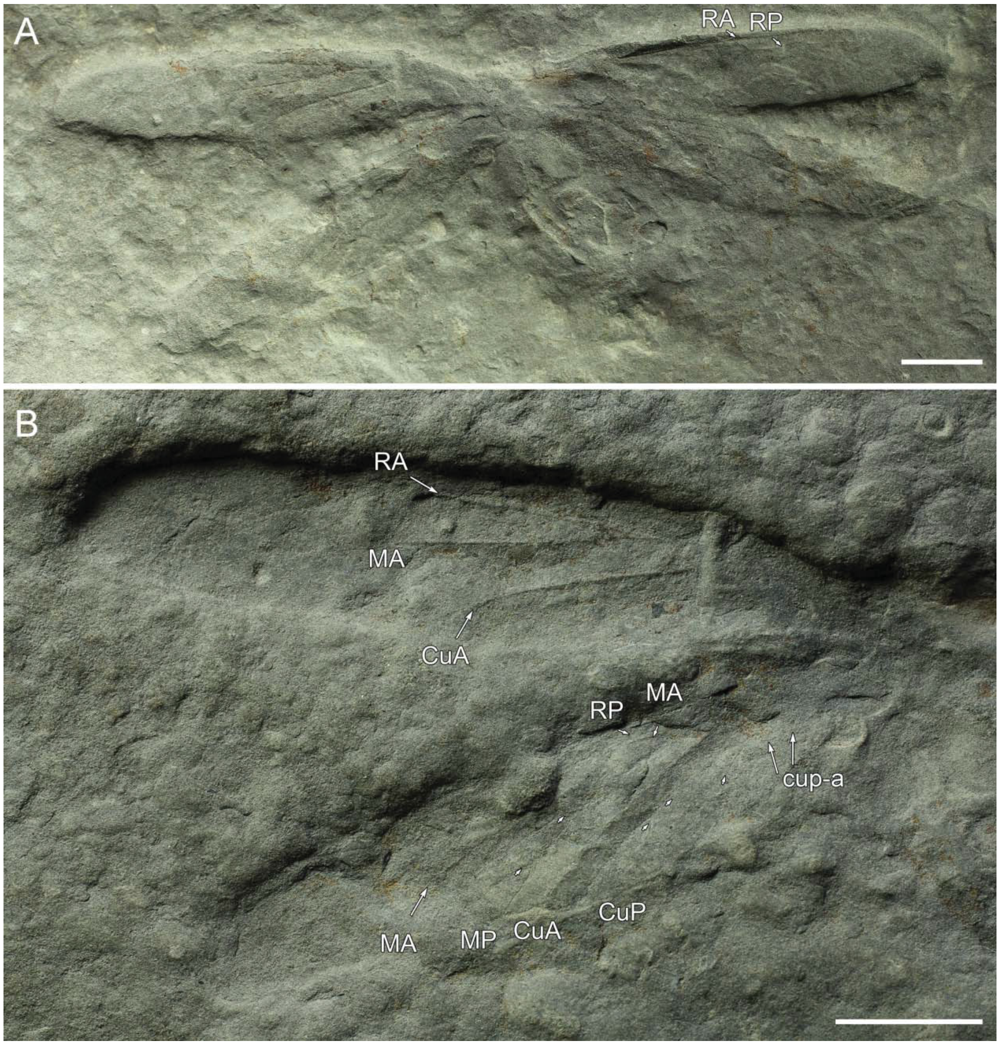

Figure 1. *Brodia jogginsensis* sp. nov., holotype, NSM008GF031.092, stored in the Joggins Fossil Centre, Joggins, Nova Scotia, Canada. **A,** photograph of habitus. **B,** photograph of left forewing and hind wing. Scale bars = 10 mm.

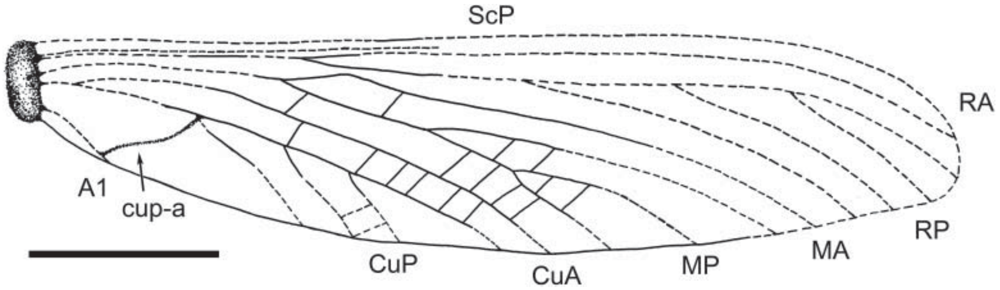

Figure 2. *Brodia jogginsensis* sp. nov., line drawing of hind wing venation, reconstructed from holotype. Scale bar = 10 mm.

neutral RP diverges from RA before mid wing running parallel forming rather broad area between RA and RP (visible on right forewing); distal part of RP posteriorly pectinate (poorly preserved); base of M running close to radius in basal part, convex MA simple, strongly approximate RP, not touching (visible on left hind wing); concave MP with three anterior terminal branches (preserved on left hind wing); several simple oblique crossveins between branches of MP, between MP and CuA, and between CuA and CuP; convex CuA simple; concave CuP running parallel along CuA, posteriorly pectinate ending with four branches (hind wing); anal area strongly reduced, single anal vein (poorly visible). Abdomen slender with 3 (?4) basal segments (poorly preserved).

Dimensions. Forewing (right) length *c*. 53 mm, width *c*. 10.4 mm; hind wing (right) length *c*. 50 mm, width *c*. 11.5 mm.

Remarks. In spite of the somewhat poor preservation, the outstretched and nearly homonomous elongate wings with markedly reduced anal areas as well as three prominent convex simple veins RA, MA and CuA clearly correspond to the extinct orders Megasecoptera and Palaeodictyoptera. The presence of shortly petiolate wings, a broader costal area in the basal part and a multi-branched MP support placement in the megasecopteran family Brodiidae (Carpenter 1992). This family currently comprises three genera: *Brodia* Scudder, 1881, from the Langsettian–Duckmantian of Coseley, Staffordshire, England; *Eubrodia* Carpenter, 1967, from the Moscovian of Illinois, USA; and *Pyebrodia* Brauckmann & Herd, 2003, from the Moscovian of Steinbruch im Piesberg near Osnabrück, Germany (Carpenter 1967; Brauckmann & Herd 2003).

Pyebrodia is distinctly smaller, with the anterior margin bending at about mid wing, MA running remotely from RP, and MP deeply bifurcated; all of these characteristics are different in the specimen studied herein. Re-examination of the type species housed at MSO reveals that *Pyebrodia martinsnetoi* clearly lacks the diagnostic characters for attribution to Brodiidae, i.e. the costal margin is markedly concave, ScP is well separated from the costal margin ending in wing apex, and MA does not approximate RP behind the division of MA and MP. In addition, the preserved part of the wing is about half the size of *Brodia priscotincta*. We assume that this pattern of venation corresponds with Palaeodictyoptera, with attribution close to Breyeriidae (e.g. *Breyeria* Borre, 1875) or the very specialized Spilapteridae (e.g. *Delitzschala* Brauckmann & Schneider, 1996). However, the familial attribution remains uncertain due to the fragmentary preservation of this fossil, which is lacking the basal parts of its wings. Another species, *Pyebrodia janseni* Zessin, 2006, clearly lacks the diagnostic characters for placement into Megasecoptera as both pairs of wings are noticeably heteronomous with an extremely broad anal area in the hind wings (see Zessin 2006, p. 39, figs 2, 3), unlike other members of this group. *Pyebrodia janseni* shares the main diagnostic characters of the palaeodictyopteran family Breyeriidae, similar to *Breyeria* or *Hasala* Brauckmann, 1995. Therefore, we propose to transfer *Pyebrodia janseni* Zessin, 2006 into Breyeriidae as *Breyeria janseni* (Zessin, 2006) new. comb. For the latest data on Breyeriidae, see Prokop *et al.* (2013). Brauckmann *et al.* (2009) synonymized *Breyeria brevis* Brauckmann & Herd, 2003 with *Pyebrodia martinsnetoi* Brauckmann & Herd, 2003.

Eubrodia shares with *Brodia jogginsensis* sp. nov. an anteriorly pectinate MP and reduced anal area but differs in that the wings are markedly broader behind the mid wing. The markedly elongate and petiolate wings that are widest well before mid wing, together with numerous rather weak and simple transverse crossveins (preserved on the right hind wing), indicate that the assignment to the genus *Brodia* is appropriate. Based on an evaluation of all available material, Bolton (1921) considered in his revision of Brodiidae that the intraspecific variability in the venation of *B. priscotincta* occurs in two or three branched MP and simple or distally forked CuP (see also Laurentiaux 1953). Carpenter (1967) considered the genus *Brodia* as monotypic and proposed the synonymy of *B. furcata* Handlirsch, 1919 with *B. priscotincta* Scudder, 1881, suggesting larger intraspecific variability in the venation for this species.

Brodia jogginsensis sp. nov. and *B. priscotincta* have similar wing sizes, but the wings of *B. priscotincta* are

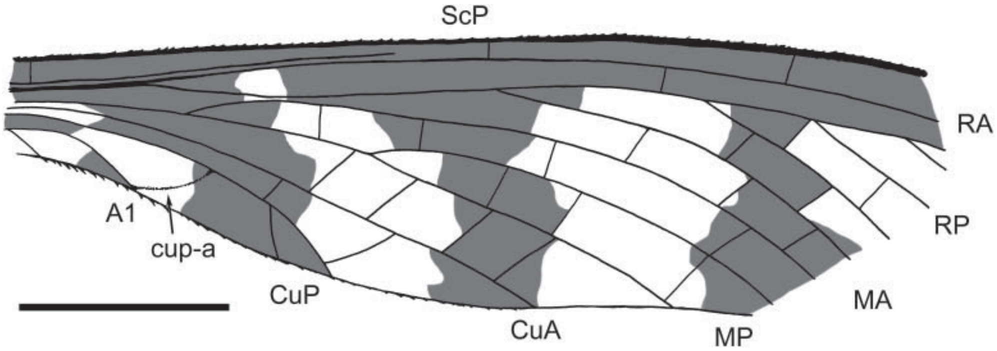

Figure 3. *Brodia priscotincta* Scudder, 1881, line drawing of holotype wing venation. Scale bar = 10 mm.

more slender close to the wing base. CuA and CuP in *B. jogginsensis* sp. nov. run closely parallel to the posterior wing margin instead of the distally diverging veins as in *B. priscotincta*. Another difference is that CuP ends with four posterior branches in *B. jogginsensis* sp. nov. Our reexamination of the holotype and of additional specimens of *B. priscotincta* confirms a clear terminal bifurcation of the CuP vein (see Figs 3, 4), unlike the previous cumulative drawing (Carpenter 1967, text-fig. 4A) that was based on specimens housed in the MCZ collection. In fact, the cumulative drawings based on several specimens with partly different vein patterns appear slightly misleading. *Brodia jogginsensis* sp. nov. and *B. priscotincta* (NHMUK 2962) both have three-branched MP, but the two differ in the reversed arrangement of pectination. In addition, the same specimen has a two-branched CuP and a markedly elongated anal area compared to *B. jogginsensis*. The separation of *B. jogginsensis* from *B. priscotincta* is clearly demonstrated.

The terminally forked CuP and the three-branched MP are also shared by *Brodia* and *Eubrodia* as proposed by Carpenter (1967). We supplement our study with drawings and photographs of the most complete and available specimens of *Brodia* and *Eubrodia* (Figs 3–6) because of inconsistencies of the drawings from the previous authors as indicated by Carpenter (1967).

Genus ***Eubrodia*** Carpenter, 1967

Type species. *Eubrodia dabasinskasi* Carpenter, 1967.

Eubrodia dabasinskasi Carpenter, 1967
(Figs 5, 6)

Material. FMNH PE 32010 (formerly H 460 in the Herdina Collection), preserved in a nodule, a negative of a nearly complete wing lacking part of the costal area close to the base and faintly preserved apical area along the posterior wing margin. Strip mines #1-6, N of Coal City, Grundy County, Illinois, USA. Late Carboniferous, Pennsylvanian, Moscovian (Westphalian C/D), Francis Creek Shale Member, Carbondale Formation.

Description. Nearly complete wing lacking the most apical part, wing membrane with dense pattern of irregular cells and crossveins (see Fig. 6C); costal margin nearly straight, bearing prominent irregular tubercles; short ScA emerging basally close to ScP, which diverges obliquely towards costal margin; humeral crossvein preserved between costal margin and ScP; concave ScP nearly straight, merging to costal margin near mid wing; RA+RP slightly bowed in basal part; RP diverges from RA approximately in the basal third of wing, ending pectinate with three posterior branches; M basally well separated from RA+RP; division of MA and MP 4.5 mm from base, convex MA diverges from MP directed towards RP, but not touching, simple MA reaching posterior margin; concave MP anteriorly pectinate ending with four terminal branches; division of cubital veins located close to wing base; single convex CuA, concave CuP running parallel to CuA ending with two branches; single anal vein reaching posterior margin close to wing base; specialized crossvein (cup-a) connecting veins CuP and A1; posterior wing margin with small basally stout spines distinctly preserved in basal part of wing.

Dimensions. Preserved wing length 51.3 mm, estimated wing length 54 mm, maximum width 16.1 mm.

Remarks. The described specimen of *Eubrodia dabasinskasi* corresponds well with the venation of the holotype, showing a more complete wing base with a ScA vein reaching the costal margin next to the humeral crossvein and bearing a similar pattern of dense irregular crossveins forming small cells of the so-called archedictyon (see Fig. 6C). The tiny differences in the terminal branches of MP possibly result from incomplete preservation in the most apical part (see Fig. 6A). Our observation of an additional illustrated specimen of *Eubrodia dabasinskasi* (HTP 433, Piecko Collection) from Carpenter & Richardson (1971, fig. 12) shows a wider anal area with clearly at least two posterior

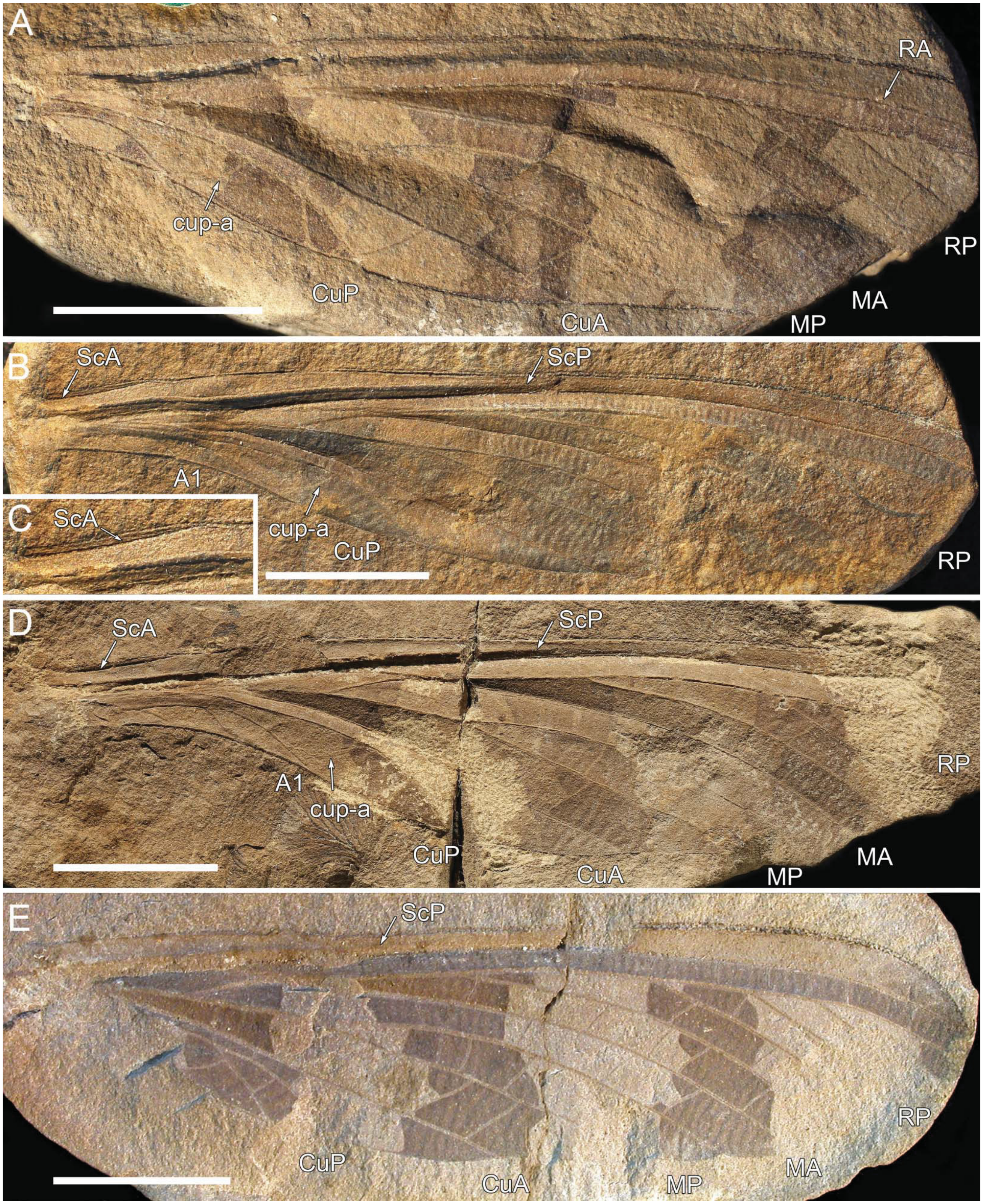

Figure 4. *Brodia priscotincta* Scudder, 1881, photographs of wings. **A,** holotype NHMUK. I3996. **B, C,** NHMUK I2961; **B,** complete wing; **C,** detail of anterior wing base. **D,** MCZ 5842. **E,** BU 686. (**A–C,** © The Natural History Museum, London). Scale bars = 10 mm.

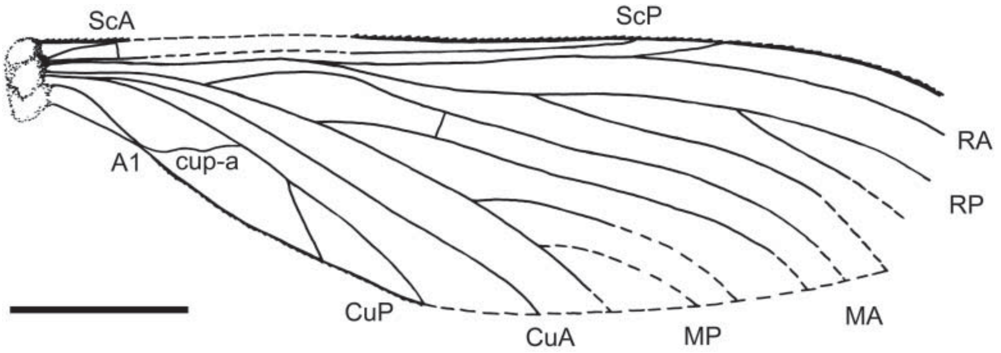

Figure 5. *Eubrodia dabasinskasi* Carpenter, 1967, line drawing of wing venation on FMNH PE 32010. Scale bar = 10 mm.

branches reaching the posterior wing margin in contrast to the holotype. Nevertheless, the specimen described here has the anal vein simple only preserved. Furthermore, our examination reveals the presence of a specialized crossvein (cup-a) between CuP and the anal vein, a trait shared with *Brodia priscotincta*. This structure is probably a reinforcement of this area and can be regarded as a possible autapomorphy of Brodiidae.

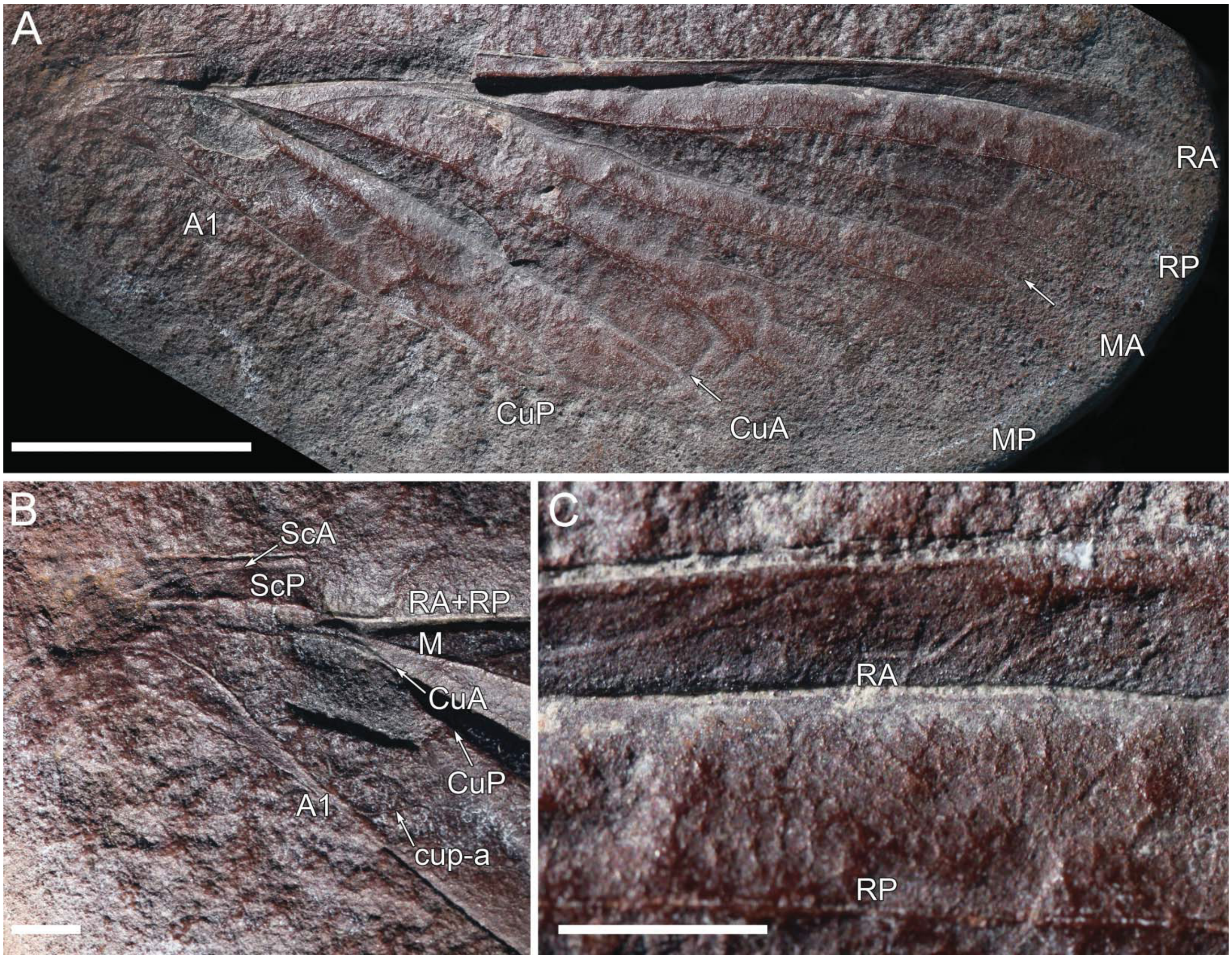

Figure 6. *Eubrodia dabasinskasi* Carpenter, 1967, photographs of FMNH PE 32010. **A,** complete wing; **B,** detail of wing base; **C,** detail of wing membrane area along anterior wing margin close to apex with pattern of irregular cells. Scale bars: A = 10 mm; B, C = 2 mm.

Discussion

Remarks concerning the wing morphology of Brodiidae

Wings in Brodiidae are nearly homonomous (hind wings slightly broader than forewings), with conspicuous corrugation of longitudinal veins that are possibly responsible for marked taphonomic deformations of areas, particularly in the anterior part of wings, especially compaction. Darkly pigmented stripes with an intermediate hyaline membrane in both wing pairs occur in *Brodia priscotincta* only. Simple and straight crossveins between longitudinal veins are widely spaced and irregularly distributed in *Brodia*, while in *Eubrodia* an archedictyon, a dense meshwork of crossveins, is present. Petiolate wings of Brodiidae bear a markedly reduced venation with a single short pectinate anal vein (Figs 4, 6).

The anterior wing margin is basally formed by costal veins CA+CP and subsequently reinforced distally by a progressive merge of subcostal vein ScA (see Fig. 4B, C) (Kukalová-Peck 1991). This prominent reinforcement of the anteroapical wing edge likely provided additional stiffness for the markedly elongated wing in contrast to other megasecopterans. The costa, particularly in the apex, bears numerous small knob-like or elliptical protuberances (tubercles), perhaps representing specialized spines with possible sensory receptors. Bolton (1921, p. 60) considered these spinules as modifications of longer hair-like sensilla (= 'macrotrichia'), following the view of Tillyard (1918). These structures are known also in other members of Palaeodictyopterida (e.g. Anchineuridae, Namuroningxiidae), and some members of modern insects like Odonata etc. (e.g. Fraser 1942; Carpenter 1963; Prokop & Ren 2007; Prokop *et al.* 2016b). The basal part of the posterior wing margin shows prominent stout spines projecting apically as noted by Carpenter (1967). Two specimens assigned to *B. priscotincta* (NHMUK I2961 and MCZ 5842, respectively from the Bolton and Scudder collections) show more completely preserved basal parts, with unambiguous evidence of well-developed and elongated ScA reaching the costal margin with marked widening of the wing (Fig. 4B, C). This character probably corresponds to "a slight hump-like elevation" close to the wing base noticed by Bolton (1921, p. 60) and subsequently overlooked and doubted by Carpenter (1967, p. 72). However, the basally separated ScA vein is scarcely documented in other Megasecoptera with the exception of *Engisoptera simplices* Kukalová-Peck, 1975 where the corresponding vein is connected basally to ScP and marked as the postcostal vein (Kukalová-Peck 1975, fig. 11). The latter author considered its homology to the costal brace of Ephemeroptera. For instance, our re-examination of *Mischoptera nigra* (Brongniart, 1893) confirms the absence of this trait but it is rather common in members of Palaeodictyoptera (see Kukalová-Peck & Richardson 1983; Engel *et al.* 2013). This markedly convex vein most likely supported the leading edge of the forewing to avoid flattening and bending (Wootton 1981). The ScP vein, slightly undulated basally, is prominently concave and runs towards the costal margin behind the mid wing. Our re-examination of Scudder's specimens 5842 and 5843 housed in MCZ supports the interpretation proposed by Bolton (1921, p. 60) that the ScP vein fails to reach the costal margin and disappears in the costal area. This is in contrast to Carpenter (1967, text-fig. 4A) who considered that the ScP merges to the costal margin, as shown in his drawing. On the other hand, Carpenter indicated that the course of the ScP vein in the distal part of wing runs closely parallel to the anterior margin formed by CA+CP+ScA. The costal area is relatively broad in Brodiidae, which is a trait that occurs in several megasecopteran families, such as Aykhaliidae, Brodiopteridae and Sphecopteridae (Pecharová *et al.* 2015a, b). On the other hand, there is a tendency for a narrower costal area in other megasecopteran groups known from younger strata (Carpenter 1992). In addition, our re-examination of *B. priscotincta* specimen MCZ 5842b reveals the presence of the short cross-vein connecting veins MA and RP in their closest approximation known in other megasecopteran families (e.g. Aykhalidae and Bardohymenidae). Thus, either the close approximation of veins MA and RP or their short connection can be a variable character, as was demonstrated by Pecharová *et al.* (2015b).

Species of Brodiidae have an extremely reduced anal area formed by a single pectinate vein with posterior branches, as in *Brodia priscotincta*, that reaches the posterior wing margin close to the wing base. The same vein in *Eubrodia* is only simple or distally twigged. Our re-examination of several specimens of *B. priscotincta* reveals the presence of a specialized crossvein (cup-a) in the form of a sigmoidal structure located between the anal vein A1 and CuP and in some specimens perhaps extending obliquely. This prominent crossvein is structurally different from the other crossveins and perhaps allows for basal reinforcement of the wing much like the anal bar (ridge) in Palaeodictyoptera (Kukalová-Peck 1991; Engel *et al.* 2013). Hence, we consider this structure as a flight functional adaptation resembling the anal bar in Palaeodictyoptera.

Wing pads in nymphs of Brodiidae and other Megasecoptera

Handlirsch (1919) described wing pads attributed to Brodiidae under the name *Brodia nympha* Handlirsch, 1919, pointing out their immaturity. Bolton (1921) considered this taxon as a junior synonym of *B. priscotincta*, with the indication '*forma juvenis*', supplementing the material with several other specimens of isolated wing pads. His

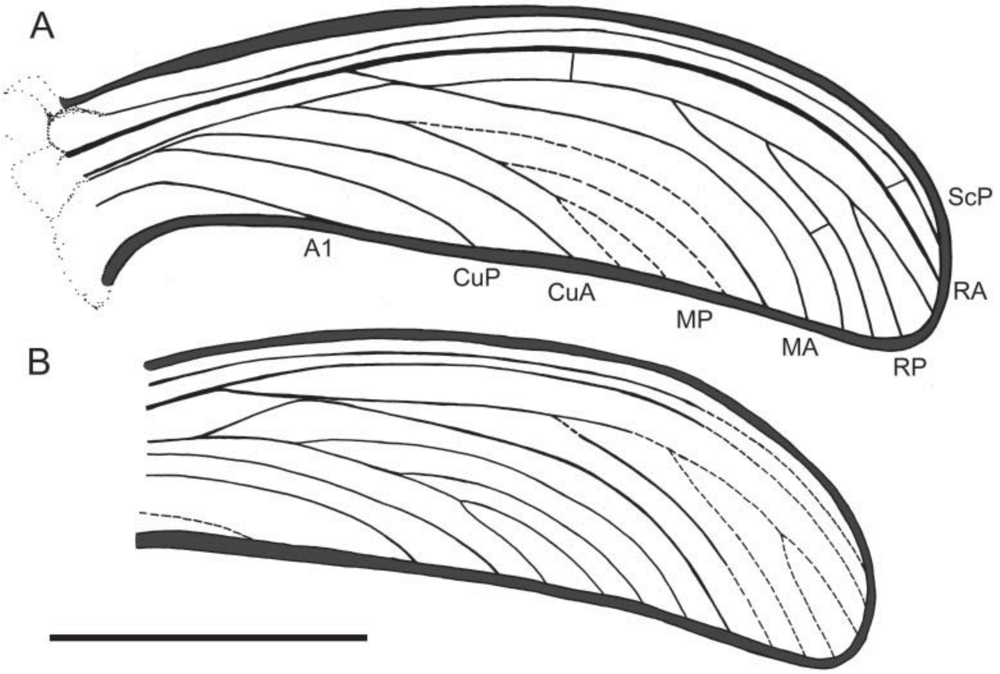

Figure 7. *Lameereites curvipennis* Handlirsch, 1911, holotype, YPM 66. **A,** line drawing of right forewing pad; **B,** line drawing of left forewing pad. Scale bar = 5 mm.

opinion was based on the assumption that the tracheae determine the position of the main longitudinal veins. While some authors have demonstrated considerable differences between the tracheation of the wing pad of immature insects and venation of the wing in the imago, extensive homologies were found in modern Ephemeroptera (Landa 1948). The record of immature palaeodictyopterans shows markedly well-developed and corrugated tracheation of nymphal wing pads comparable to the future venation in imago as was demonstrated on *Tchirkovaea guttata* Zalessky, 1931 (Tchirkovaeidae), known from the holotype from the Kuznetsk Basin in Russia and series of specimens from the Tunguska Basin (Sinitshenkova 1979).

The nymphs of Megasecoptera are more scarcely documented, known from just a few well-preserved specimens such as *Lameereites curvipennis* Handlirsch, 1911 (family indet.) and *Mischoptera douglassi* Carpenter & Richardson, 1968 (Mischopteridae), both from Mazon Creek nodules in the Pennsylvanian of Illinois (Carpenter & Richardson 1968). Carpenter (1967) first considered that *Lameereites* could belong to Brodiidae, based on similar patterns of wing pad tracheation and wing venation, but later confirmed only placement in Megasecoptera without a family assignment (Carpenter & Richardson 1968). Haug *et al.* (2016) reported and figured an additional specimen (ROM No. 45546) of a nymph from a Mazon Creek Pennsylvanian nodule, indicating a resemblance to both *Mischoptera douglassi* and *Lameereites curvipennis*. Haug *et al.* (2016) assumed on the basis of comparison of branching patterns and similar sizes that *Lameereites curvipennis* and *Mischoptera douglassi* are probably conspecific. Nevertheless, upon re-examination of the holotype of *Mischoptera douglassi* (stored in the private collection of Douglass, Western Springs, Illinois, USA), they suggested the combination ?*Mischoptera curvipennis* (Handlirsch, 1911). In contrast, we support the view proposed by Carpenter (1967) as there are several clear differences in these wing pads, all widely used in the taxonomy of Megasecoptera, such as: the division of MA/MP relative to RA/RP; the number of terminal branches of MP; the length of the anal area; as well as clearly different patterns of crossveins (see our reinterpretation below, Figs 7, 8). Therefore, we consider the above-mentioned differences in the developing tracheation of wing pads as significant traits and propose restoration of the generic name *Lameereites* for ?*Mischoptera curvipennis* as *Lameereites curvipennis* stat. rest.

Haug *et al.* (2016) indicated another specimen (YPM IP 204159) with preserved isolated wing pads and part of an attached tergum, which was also tentatively attributed to *Mischoptera*? *curvipennis*. However, this specimen clearly differs from both previously described taxa and instead shows a markedly enlarged keel in the costal area, which is characteristic for mesothoracic wing pads of early instars of Palaeodictyoptera (see Prokop *et al.* 2017).

The formation of the costal area in forewings represents a marked difference in the development of wing pads of

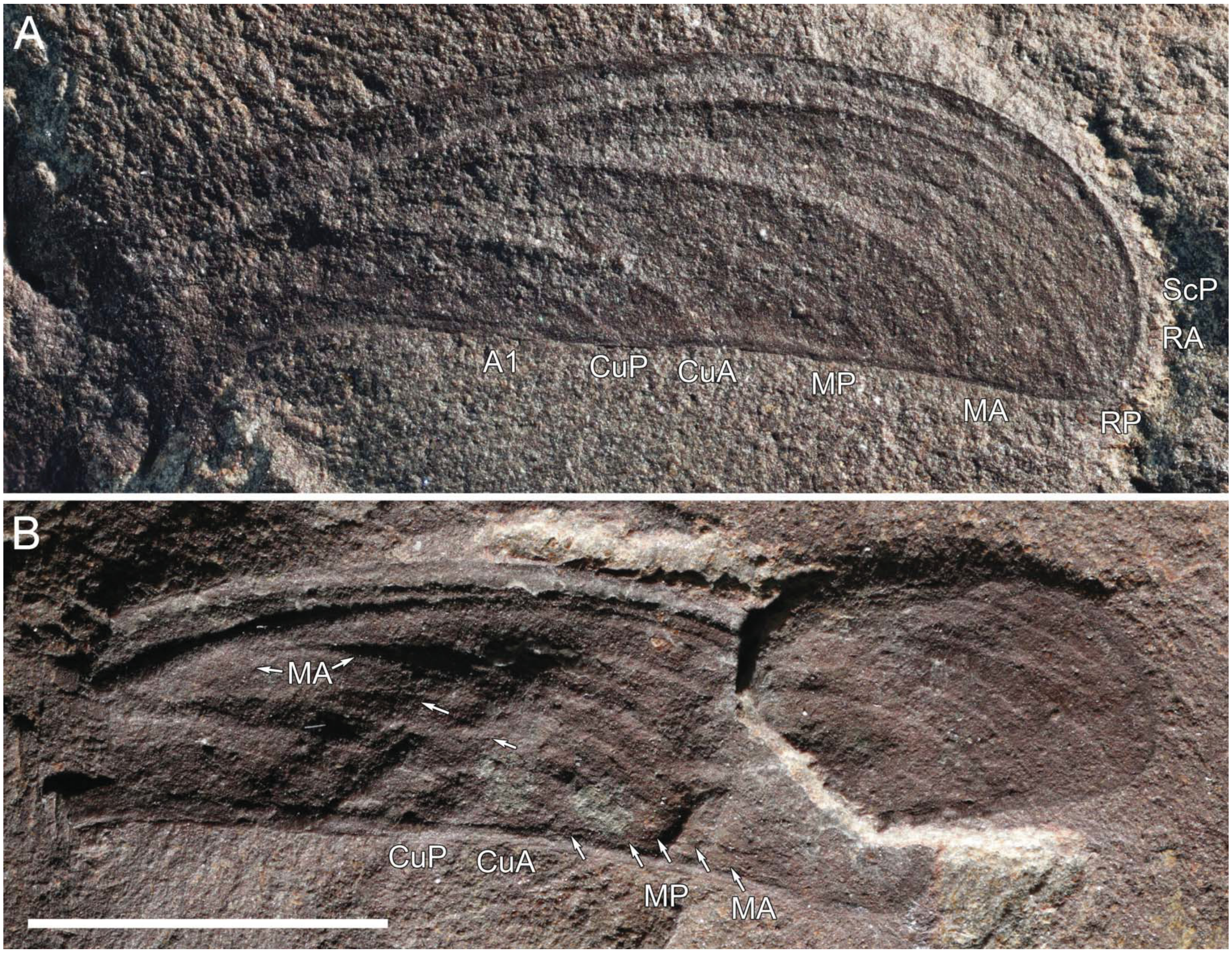

Figure 8. *Lameereites curvipennis* Handlirsch, 1911, holotype, YPM 66. **A,** photograph of right forewing pad; **B,** photograph of left forewing pad and isolated apex of hind wing pad. Scale bar = 5 mm.

immature Megasecoptera and Palaeodictyoptera; palaeodictyopterans bear a noticeably broad keel, usually as a continuation of the enlarged prothoracic winglets. There is no such extension in megasecopterans. Instead of prothoracic winglets, the available specimens of *Mischoptera douglassi* show long laterally protruding spines with most likely a protective function, and the first pair of wing pads has a slightly broader costal area only.

Based on such a fragmentary record of nymphal instars, the steps in growth of wing pads during postembryonic development cannot be estimated conclusively. However, the three documented specimens from Mazon Creek attributed to *Mischoptera douglassi* represent at least two different instars on the basis of their body sizes: the younger instar (FMNH PE31976), reaching a body length of about 38 mm, and two older instars (Douglass Collection 39 and HTP 1232) with body lengths of about 50 mm. In comparison, the body length of an adult specimen of *Mischoptera nigra* (Brongniart, 1885) known from the Commentry Basin in France (MNHN R51057) reaches 88 mm. Considering the position of the wing pads in these immature specimens, the older instars confirm the small increase of wing pad length, as well as a slightly expanded position remote from the thorax.

Re-examination of other isolated, slightly curved, immature wing pads in fossils from the NHMUK collection figured by Bolton supports their placement in Megasecoptera because of their slender shape, marked corrugation, with elevated convex veins of RA, MA and CuA, and strongly reduced anal area (Fig. 9). However, the combination of some traits – such as the close approximation of MA and RP, multiple MP and organization of the cubital and particularly the anal area – supports their placement in Brodiidae. Nevertheless, we prefer to follow Carpenter's (1967) example by not assigning them to a particular family as the number of terminal branches can increase during wing development. The enlarged outer margin of these wing pads is clear evidence for the presence of cuticular sheaths as known, for instance, in modern mayflies (Ephemeroptera).

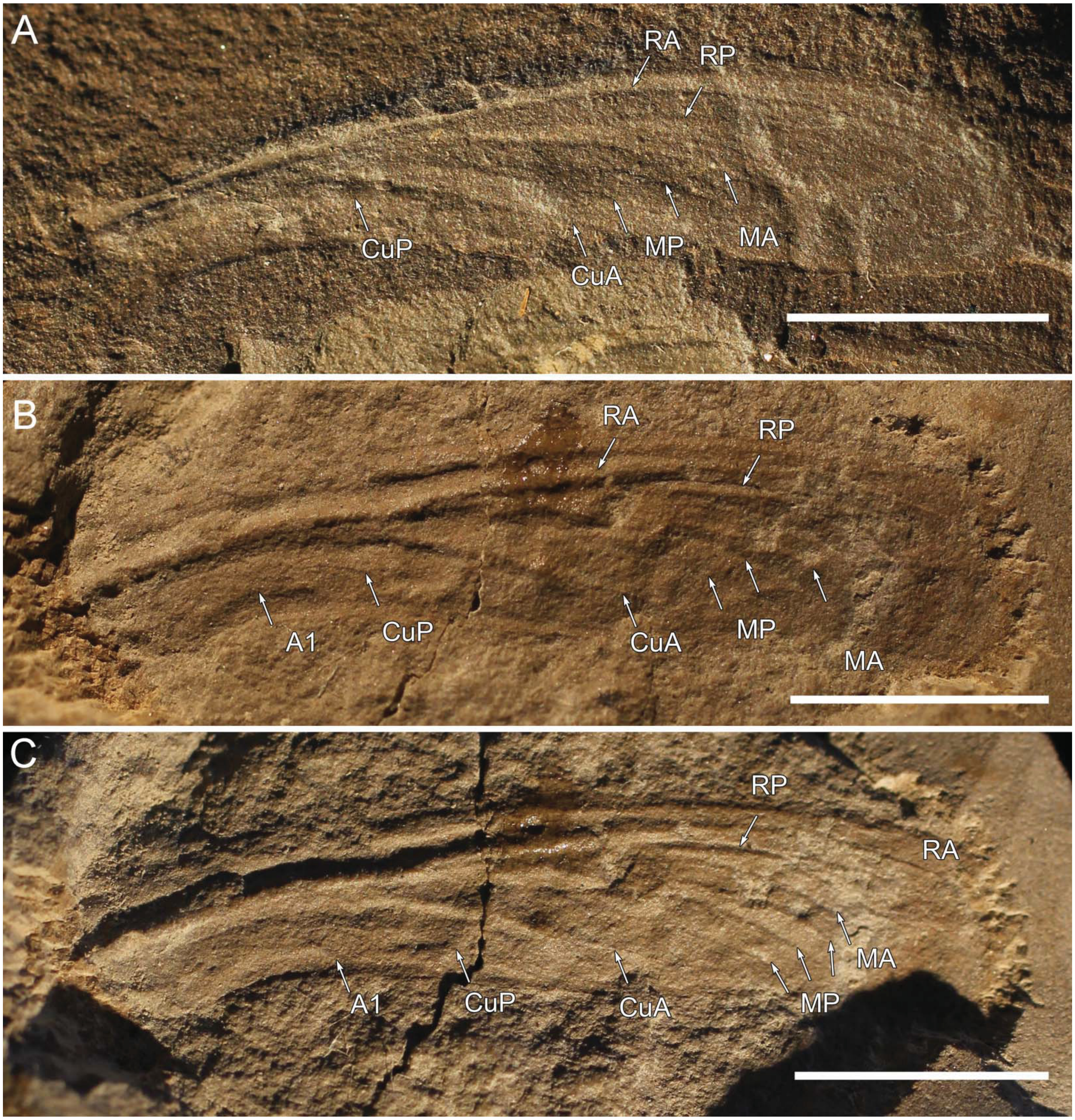

Figure 9. ?Brodiidae gen. et sp. indet., photographs of supposed immature wing pads described by Bolton (1921). **A,** NHMUK I1563 (part); **B, C,** NHMUK I2966 (part and counterpart). (© The Natural History Museum, London). Scale bars = 5 mm.

Morphology and adaptations in immature stages of Megasecoptera

On the basis of the new observations, we can clarify aspects of the development, morphology and function of some significant body structures of nymphal stages with respect to adult megasecopterans:

1. A hypognathous head of triangular shape with bulging eyes and particularly well-developed haustellate mouthparts in the form of a rostrum are present in the holotype of *Lameereites curvipennis* and a supplementary specimen of an early instar of *Mischoptera douglassi* (FMNH PE31976). The latter shows the original position of the head capsule with rostrum to the dorsoventral body axis, which forms an acute angle of approximately 60° (Fig. 10A). Haustellate mouthparts, formed by a relatively short and basally stout beak with a strongly sclerotized

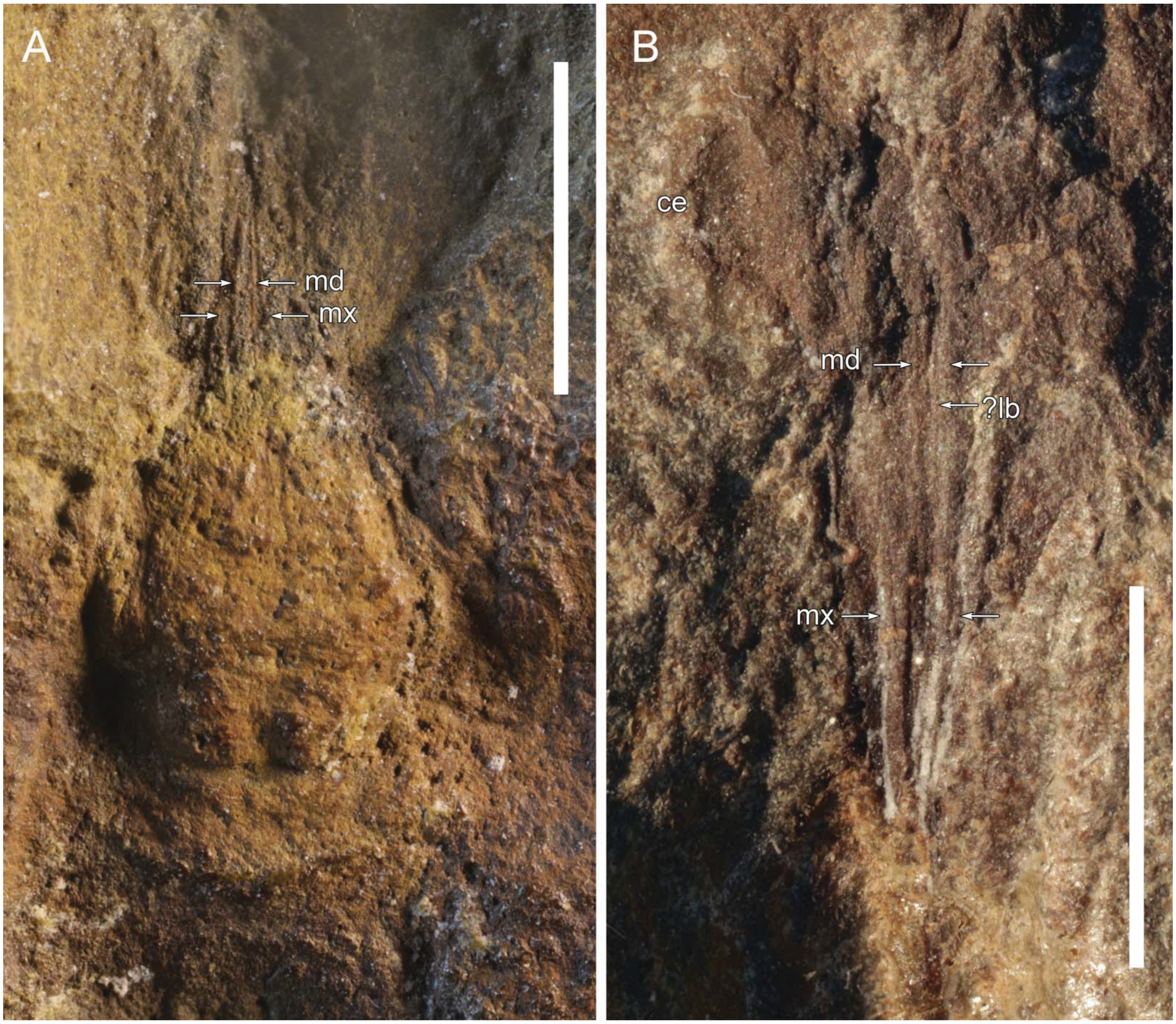

Figure 10. Photographs of heads with mouthparts. **A,** *Mischoptera douglassi* Carpenter & Richardson, 1968, FMNH PE31976. **B,** *Lameereites curvipennis* Handlirsch, 1911, holotype, YPM 66. Scale bars = 3 mm.

mandibular and large maxillar stylets covering the labium, are partially preserved in *L. curvipennis* (Fig. 10B). This type of rostrum occurs in some adults of megasecopteran families including Aykhalidae, Mischopteridae and Protohymenidae, and supports a similar diet of nymphs and adults (Carpenter 1992). Nevertheless, another significantly elongated type of rostrum is known in members of Brodiopteridae that lived at the same time in taxa from Xiaheyan in China, supporting the size disparity of feeding adaptations among megasecopterans (Pecharová *et al.* 2015b, fig. 10; Prokop *et al.* 2016b). Similarly, a rostrum of short type occurs in some palaeodictyopterans, such as members of Spilapteridae and diaphanopterodeans, for instance Parelmoidae (e.g. Shear & Kukalová-Peck 1990; Rasnitsyn & Novokshonov 1997; Li *et al.* 2013). Hence, the disparity of rostrum-like mouthparts corresponds to specialization for different plant sources. Such plant–insect interactions are also recorded as piercing and sucking damage as stylet probes on marattialean tree ferns or punctures on Cordaite seeds (Sharov 1973; Labandeira & Philips 1996; Shcherbakov *et al.* 2009). Antennae of *Lameereites* are clearly of filiform type, with basal segments such as the scapus and pedicellus not clearly discernable while the flagellum is more clearly preserved in the distal part. The length of antennae probably slightly extends beyond the tip of the rostrum.

2. The thorax with shorter prothorax and longer meso- and metathorax was carrying long spines emerging from nota, as known in the holotype of *Mischoptera*

douglassi (Carpenter & Richardson 1968, text-fig. 2). The prothorax of *M. douglassi* (FMNH PE31976) shows only weakly preserved pronotum edges with traces of lateral spines faintly preserved; the meso- and metanotum are lacking these structures. Similarly, the tracheation of the wing pads in this specimen is weakly preserved and does not allow for the recognition of the original course of the tracheae, probably due to their immaturity. On the other hand, well sclerotized lateral spines on each side of the pronotum are clearly preserved in the supplementary specimen HTP 1232. Unfortunately, the thoracic segments in *Lameerites* are not preserved. The elevated and sclerotized basal joint between the wing pad and thorax in *L. curvipennis* supports the presence of a muscle attachment at least at the base of radius, which probably allowed limited movement of the wing pad. The well-developed, broadly enlarged outer margin supports the presence of the cuticular sheaths. However, our re-examination of the wing pads in *L. curvipennis* reveals a partly different pattern of tracheation in contrast with the composite drawing given by Carpenter & Richardson (1968, pl. 25). The difference lies in the division of RA and RP (which was shifted distally), and the number of terminal branches in MA, MP and CuA. We assume that MA is simple and the supposed posterior branch of MA is in fact the anterior branch of MP; the MP is then four-branched. The CuA seems to be simple and only a single anal vein is discernable, in contrast to the previous study. Our interpretation was cross-checked by the corresponding left and right forewing pads with more clearly developed tracheation against the more incomplete hind wing pads (Figs 7, 8). The alternation of main convex and concave tracheae is easily discerned and thus corresponds to what is known for immatures of Palaeodictyoptera. However, the faint preservation of these tracheae along the posterior wing margin allows for supplementary terminal branching during subsequent development. Finally, our new interpretation of the pattern of tracheation in *L. curvipennis* seems to correspond with the venation in Brodiidae and particularly in *Eubrodia*, as considered by Carpenter (1967).

3. In the available specimens the abdomen is fragmentary or completely missing as in *Lameerites*. Thus, our knowledge is mainly based on the structures documented in the holotype of *Mischoptera douglassi* and additional remarks on adaptations seen in other available specimens. A specimen of a young nymph of *M. douglassi* (FMNH PE31976) bears part of the abdomen with nota (I–VIII), showing an internal view with several holes arranged in lines along the posterior margins of segments III–VII. We assumed that these structures probably correspond with the cross sections of spines. These spine rows were documented in the holotype of *M. douglassi* by Carpenter & Richardson (1968) who also found homologous structures on adults of *Mischoptera nigra* (Brongniart, 1885) from the Moscovian of the Commentry Basin in France. Kukalová-Peck (1972) confirmed the presence in *M. douglassi* of such remarkably preserved rows of seven stout spines along the posterior margin of each abdominal tergite with the exception of last two segments. Moreover, the same author indicated that the posterior margin of terga in *Mischoptera douglassi* carried long integumental projections covered with setae (Kukalová-Peck 1978). Rows of spines or tubercles along the posterior edges of abdominal nymphal terga are common in some groups of modern insects including Odonata and Plecoptera. Thus, we assume that these spines had a protective function against predators.

Conclusions

The record of Pennsylvanian Brodiidae known from early Langsettian to Moscovian is revised. *Brodia jogginsensis* sp. nov. presents another link between known species from Euramerica, supporting a wide distribution of Brodiidae during the Pennsylvanian. *Brodia jogginsensis* sp. nov. represents the first formally described megasecopteran from the Langsettian (Bashkirian) Joggins Formation in the Maritimes Basin of Nova Scotia, Canada. In spite of its rather poor preservation, the holotype of this species with outstretched fore- and hind wings provides the first evidence since Scudder's time for nearly homonomous wings in Brodiidae. Furthermore, our re-examination of *Pyebrodia* based on the type species *Pyebrodia martinsnetoi* from the Moscovian of Steinbruch im Piesberg near Osnabrück reveals the lack of the main diagnostic characters for attribution to Brodiidae as the costal margin is markedly concave, ScP well separated from costal margin ending in wing apex, and MA is not approximating RP behind the division of MA and MP. In addition, the preserved part of the wing is about half the size of *Brodia priscotincta*. Therefore, we consider *Pyebrodia* as member of Palaeodictyoptera, most probably belonging to Breyeriidae, and thus Brodiidae consist of only two genera: *Brodia* and *Eubrodia*. *Pyebrodia janseni* Zessin, 2006 is transferred to *Breyeria* (Breyeriidae), resulting in the new combination *Breyeria janseni* (Zessin, 2006). An additional specimen of *Eubrodia dabasinskasi* stored at the FMNH is described to supplement the holotype in the private Douglass collection at Western Springs, Illinois.

Knowledge acquired through the study of isolated immature wing pads of Brodiidae as well as other available immature megasecopterans is revisited. Based on our re-evaluation of wing pad characters we propose to restore the generic name *Lameereites* for ?*Mischoptera* *curvipennis*. The new interpretation of forewing pad tracheation in *Lameereites curvipennis* is figured and its pattern matches Brodiidae, as previously considered by Carpenter & Richardson (1968). All studied specimens of megasecopteran nymphs support the growth and development of their wing pads in the sheaths. However, in contrast to previous studies, there is no clear evidence for gradual elongation and straightening of the wing pads during postembryonic development. Therefore, we assume that wing development in Megasecoptera was allometric and similar to Palaeodictyoptera (Carpenter & Richardson 1968; Prokop *et al.* 2016a, 2017). Wing morphology and ontogeny supports a close relationship between Megasecoptera and Palaeodictyoptera.

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Článek 6

Prokop, J., Pecharová, M., Nel, A., Hörnschemeyer, T., Krzemińska, E., Krzemiński, W. & Engel, M. S. 2017. Paleozoic nymphal wing pads support dual model of insect wing origins. *Current Biology* 27: 263–269.

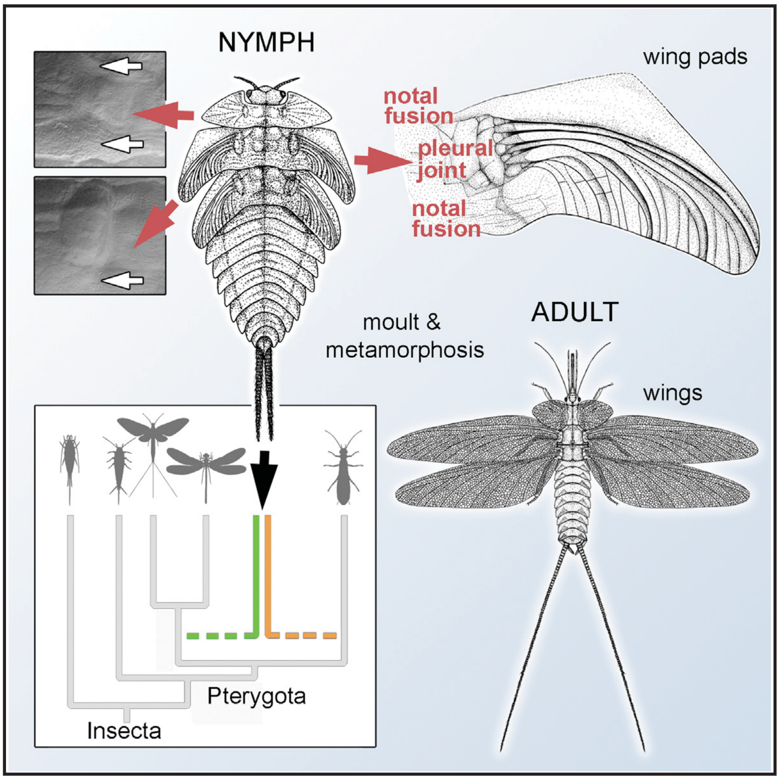
Podíl autorů: JP: korespondenční autor, texty, studium materiálu, fotografie, snímky SEM, snímkování materiálu na mikro-CT; **MP**: kresby, schéma, část fotografií, interpretace křídelní žilnatiny; **AN**: úprava textů, diskuze; **TH**: úprava textů, vytvoření 3D modelu z mikro-CT dat; **EK** a **WK**: poskytnutí části materiálu, informace o lokalitě, úprava textů; **ME**: úprava textů, jazyková korekce, diskuze.

Report

Current Biology

Paleozoic Nymphal Wing Pads Support Dual Model of Insect Wing Origins

Graphical Abstract



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In Brief

Prokop et al. demonstrate that the three pairs of nymphal wing pads in Carboniferous species of the extinct insect order Palaeodictyoptera were medially articulated to the thorax by the sclerites and also markedly fused anteriorly and posteriorly to the notum. This evidence corroborates the dual model for insect wing origins.

Highlights

- Palaeodictyopteran wing pad joints corroborate dual origin of insect wings
- Three pairs of wing pads were medially articulated and markedly fused with notum
- Wing pads were functionally unable to perform active flight due to limited divergence
- Arrangement of wing pad axillary sclerites corresponds to Hamilton's ancestral model

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Current Biology
Report

Paleozoic Nymphal Wing Pads Support Dual Model of Insect Wing Origins

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SUMMARY

The appearance of wings in insects, early in their evolution [1], has been one of the more critical innovations contributing to their extraordinary diversity. Despite the conspicuousness and importance of wings, the origin of these structures has been difficult to resolve and represented one of the "abominable mysteries" in evolutionary biology [2]. More than a century of debate has boiled the matter down to two competing alternatives—one of wings representing an extension of the thoracic notum, the other stating that they are appendicular derivations from the lateral body wall. Recently, a dual model has been supported by genomic and developmental data [3–6], representing an amalgamation of elements from both the notal and pleural hypotheses. Here, we reveal crucial information from the wing pad joints of Carboniferous palaeodictyopteran insect nymphs using classical and high-tech techniques. These nymphs had three pairs of wing pads that were medially articulated to the thorax but also broadly contiguous with the notum anteriorly and posteriorly (details unobservable in modern insects), supporting their overall origin from the thoracic notum as well as the expected medial, pleural series of axillary sclerites. Our study provides support for the formation of the insect wing from the thoracic notum as well as the already known pleural elements of the arthropodan leg. These results support the unique, dual model for insect wing origins and the convergent reduction of notal fusion in more derived clades, presumably due to wing rotation during development, and they help to bring resolution to this long-standing debate.

RESULTS

To investigate the question of wing development and potential implications for discerning their evolutionary origins, we investigated well-preserved nymphal Palaeodictyoptera. The extinct Palaeodictyoptera are among the oldest of Pterygota, appearing near the Early-Late Carboniferous boundary approximately 325 million years ago (mya) [7–9] and becoming extinct by the cataclysmic End Permian Event [2]. Palaeodictyoptera were one of a series of orders in the Palaeodictyopterida, a diverse clade of insects with permanently outstretched wings, except for Diaphanopterodea, which possessed a unique wing flexion. Palaeodictyoptera, along with their relatives within this superorder, possessed piercing-sucking mouthparts that formed a beak, which perhaps allowed them to feed on plant fluids, representing an early dietary specialization among insects [10–12]. Palaeodictyoptera had wings with a comparatively simple arrangement of veins, lacking the basal fusions between the principal longitudinal sectors, and corresponding to putative hypotheses of the overall pterygotan ground plan wing form [13]. The phylogenetic placement of this order remains unclear but is certainly among the early-diverging branches of Pterygota [14].

While palaeodictyopteran wings are commonly found in Paleozoic deposits, the wing pads of their immatures or the shed exuviae from nymphal molts are considerably scarcer in the fossil record [15]. Palaeodictyoptera are central to the debate regarding the evolutionary development of wings as their nymphal pads are unique among insects, whereby they were articulated and capable of controlled movement [16, 17]. While palaeodictyopteran nymphs historically were speculated to have been aquatic [18], current evidence rather supports common terrestrial environments for both nymphs and adults, particularly based on their feeding and respiratory structures [10, 19].

Ontogenetic evidence suggests gradual growth through numerous instars with articulated, movable wing pads, although the range of motion was limited. These were arranged along the body in younger instars and gradually straightened in later

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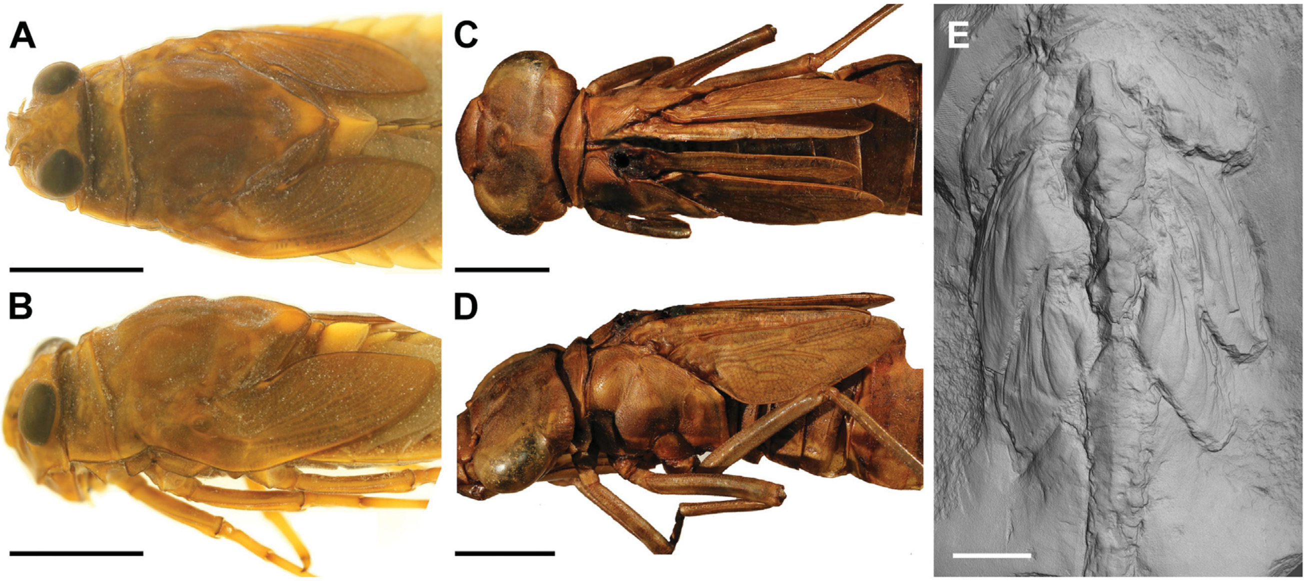

Figure 1. Position of Wing Pads from Dorsal and Lateral Views

(A and B) *Oniscigaster distans*, Oniscigastridae, Ephemeroptera, Taringamotu R., Auckland Prov., New Zealand, coll. T. Soldán, Institute of Entomology AS, České Budějovice, Czech Republic.

(C and D) *Anax junius*, Aeshnidae, Odonata, Honolulu Sandwich, Hawaii, coll. MNHN. Paris, France.

(E) Palaeodictyoptera family indet., nymph, dorsal view, ISEZ PAN IF-MP/1492/365/09 reconstructed from micro-CT data, Late Carboniferous, Bashkirian, Upper Silesian Coal Basin, Sosnowiec–Klimontów, Poland.

Scale bars of (A), (B), and (E) represent 3 mm; scale bars of (C) and (D) represent 5 mm.

stages [20, 21]. The occurrence of several subimaginal stages has also been argued for Palaeodictyoptera [20], but evidence is lacking. The forewing pads in young instars were distinguishable by their broadly triangular costal areas, as in the continuous shape of the enlarged prothoracic wing pads. These then became narrower in later developmental stages. However, the record is patchy, and evidence for gradually narrowing wing pads through successive ontogenetic stages cannot be demonstrated convincingly [19]. Data from palaeodictyopterans implicate the retention of the developing wings within exuvial sheaths until the latest instars, a pattern of development known from exopterygotes and holometabolan pupae. Thus, the growing wing pads are protected from physical damage and permit the organism to inhabit and conceal itself within small crevices without jeopardizing the developing wings. The morphology of various body parts such as the external genitalia and, notably, the pattern of wing venation in Palaeodictyoptera correspond in some traits to those of extant mayflies (Ephemeroptera) and dragonflies (Odonata) (Figure 1). While extant immatures of these latter groups show derived traits such as dorsally placed wing pads or their rotation (in Odonata), their Paleozoic stem-group relatives such as griffenflies (Protodonata) appear to have also possessed articulated wing pads [22], implying that this form in protodonatans and palaeodictyopterans is plesiomorphic.

The gradual development of movable wing pads during postembryonic development was considered primary evidence to support the “epicoxal” hypothesis of wing origins [13, 20]. However, our examination of key taxa and newly recovered material of Palaeodictyoptera testifies against the hypothesis of fully articulated wing pads and instead builds and expands upon alternative interpretations [17, 23]. This is particularly demonstrated in nymphs of *Idoptilus onisciformis* and *Rochdalia parkeri* (Figure 2), originally described with partially formed, proximal articulations while the posterior section of the developing pads were putatively fused to the scutellum [17]. Subsequent reinterpretations of the same material led to the hypothesis of fully articulated wing pads, with the articulation extending nearly to the posterior wing margin and the connection of an axillary cord to the scutellar bridge near the axillary sclerites [20]. While this was heralded as evidence for fully articulated wing pads in immature stages of all early pterygotes and the epicoxal hypothesis, evidence for the incorporation of notal elements was simultaneously suggested [23].

Here, we thoroughly revise available and new fossils of palaeodictyopteran nymphs and their exuviae to clarify morphological interpretations and to see what evidence can be brought to bear regarding wing origin hypotheses. Reexamination of nymphs of *I. onisciformis* and *R. parkeri* demonstrates the presence of a clearly discernable articulation on all three pairs of wing pads, although the prothoracic pair exhibits less-developed, movable joints. Based on our observations of well-preserved metathoracic wing pads, we can discern a clear division between a basisubcostal plate (bsc) and the remaining portion of the larger axillary plate (axp) (Figures 2A–2C). This marked separation of both plates was previously observed in adult Palaeodictyoptera [13]. The wing pads bear a notable pattern of corrugation, which probably reflects the tracheae that extend through developing insect wings and likely corresponds to the developing veins in some early pterygote groups like Palaeodictyoptera and Ephemeroptera [17, 25]. Veins in modern insects

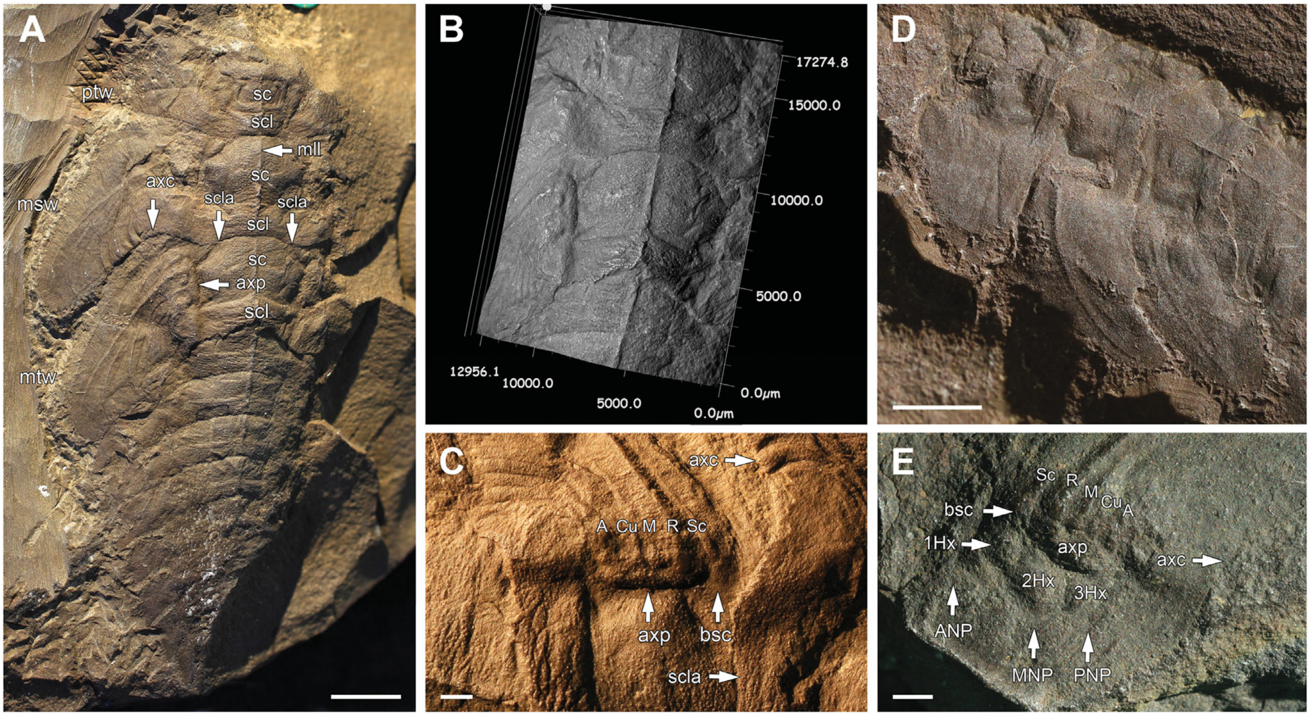

Figure 2. Wing Pads of Palaeodictyopteran Nymphs

(A–C) *Idoptilus onisciformis*, holotype NHM In 44654, Late Carboniferous, Bashkirian, Middle Coal Measures of Barnsley, UK.

(A) Habitus, dorsal view. Abbreviations are as follows: axc, axillary cord; mll, median longitudinal line; msw, mesothoracic wing pad; mtw, metathoracic wing pad; ptw, prothoracic wing pad; sc, scutum; scla, scutellar arms; scl, scutellum.

(B) 3D reconstruction of thoracic alinota with metathoracic wing pad joint relief.

(C) Detail of mesoscutum with partially developed metathoracic wing pad articulation and mesoscutellum and metascutellum broadly fused with corresponding wing pad. Abbreviations are as follows: axp, axillary plate; bsc, basisubcostale; main basal tracheae: Sc, subcostal; R, radial; M, median; Cu, cubital; A, anal.

(D) *Rochdalia parkeri* holotype MM L.11464, Late Carboniferous, Bashkirian, Lower Coal Measures, Rochdale, Lancashire, UK.

(E) ?*Idoptilus* sp., YPM IP204159, Late Carboniferous, Moscovian, Mazon Creek Lagerstätte, Illinois, USA: 1Hx, 2Hx, 3Hx, supposed axillary sclerites according to Hamilton [24]; ANP, MNP, PNP, anterior, median, posterior notal wing processes.

The scale bar of (A) represents 5 mm; the scale bar of (C) represents 1 mm; the scale bar of (D) represents 3 mm; the scale bar of (E) represents 1 mm. (Images A–C, copyright Natural History Museum, London.)

do not always follow the tracheae [26], and so tracheal positions and paths can only be used sparingly and in certain taxa when establishing identities and homologies. Here, however, these ridges, which probably correspond to the position of tracheae, do appear to correspond with later longitudinal sectors. Furthermore, our observations reveal a broad posterior connection of all wing pads to the scutellum and a connection of the axillary cord to the scutellar bridge, as mentioned for the aforementioned taxa (Figures 2A–2D). A notably broad connection between the wing pad and the scutellum is unknown in extant species of Odonata and Ephemeroptera (with the exclusion of Pannota bearing mesothoracic wing pads fused mesally to the mesonotum) but surprisingly also occurs sporadically in several groups of Neoptera, such as Dictyoptera, Plecoptera, and others where developing wing pads emerge dorsally without an articulation.

An isolated forewing pad from the Mazon Creek Lagerstätte was previously treated as a megasecopteran, *Mischoptera*? *curvipennis* (Mischopteridae) [27], but our examination supports placement in Palaeodictyoptera based on the presence of an enlarged triangular costal area and the distinctive pattern of ridges of probable tracheation. Notably, this specimen possesses a basal joint with discernable developing articulation of the wing pad (Figures 2E, 3A, and 3B), as well as a marked separation between basisubcostal and axillary plate, and three developing sclerites (1Hx, 2Hx, and 3Hx) and notal processes (ANP, MNP, PNP) with attached musculature (Figure 2E). Such an arrangement corresponds well to a proposed model of the “ancestral” pterygote [24]. Furthermore, the broad fusion of the mesoscutellum to the posterior part of the wing pad along the anal area and an axillary cord connected to the scutellar bridge corroborate those observations from *I. onisciformis* and *R. parkeri*. Accordingly, the pattern of tracheation in the Mazon Creek nymph’s wing pad markedly resembles *I. onisciformis* with the exception of a more developed cubitus posterior (CuP). This nymph could belong to the same or a related genus. However, attribution based on the pattern of ridges is limited, and we prefer to treat such isolated wing pads outside of formal nomenclatural assignments.

An incomplete exuvia of a palaeodictyopteran nymph from the Late Carboniferous of the Upper Silesian Coal Basin, Poland (No. ISEZ PAN IF-MP-1576-326-10) is detached along the median

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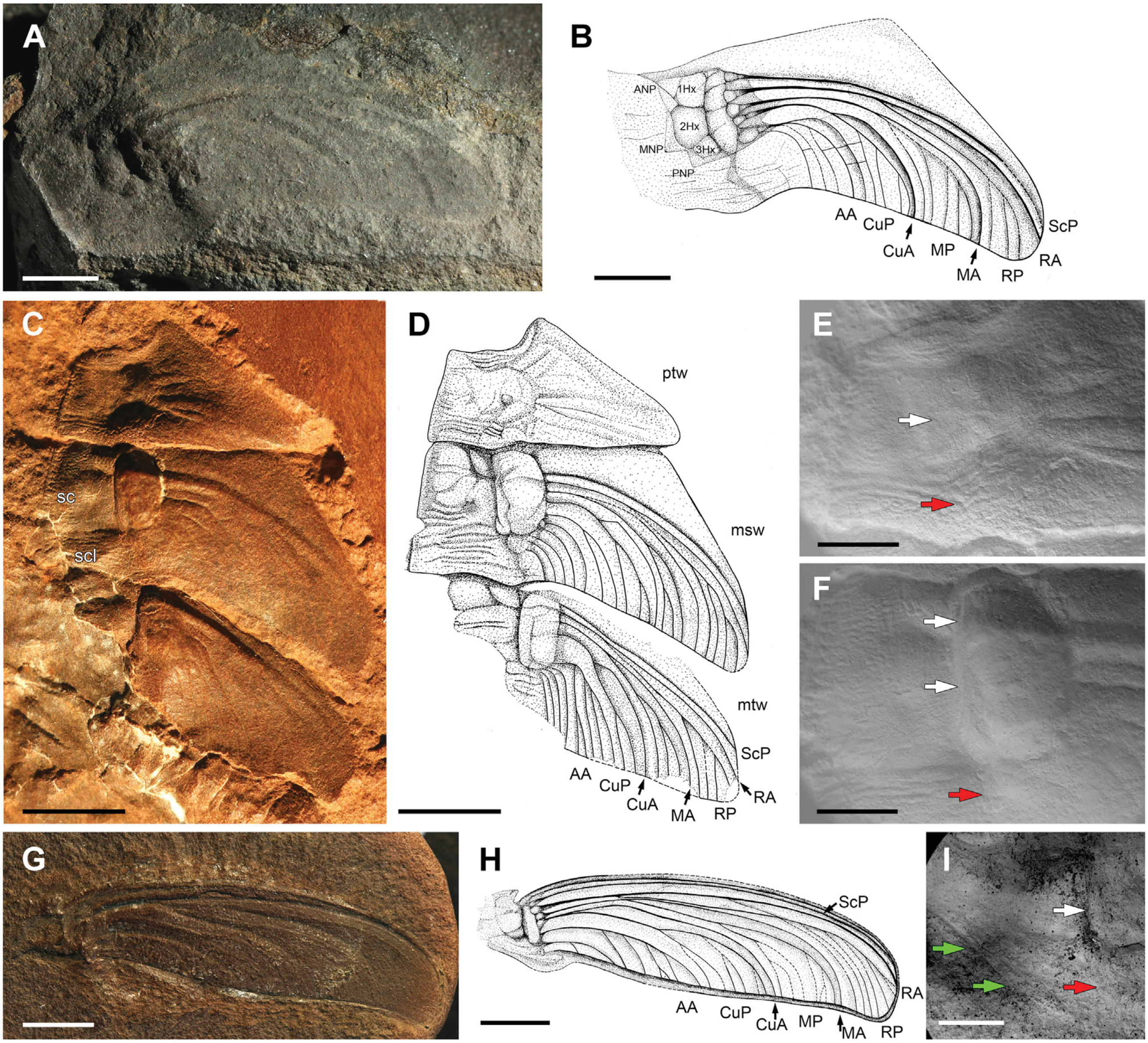

Figure 3. Wing Pads and Exuvia of Palaeodictyopteran Nymphs

(A) ?*Idoptilus* sp., YPM IP204159, Late Carboniferous, Moscovian, Mazon Creek Lagerstätte, Illinois, USA.

(B) Reconstruction of ?*Idoptilus* sp. wing pad including articulated joint, YPM IP204159 (drawn by M.P.): 1Hx, 2Hx, 3Hx, supposed axillary sclerites according to Hamilton [24]; ANP, MNP, PNP, anterior, median, posterior notal wing processes; tracheae: ScP, subcostal posterior; RA/RP, radial anterior/ posterior; MA/MP, median anterior/posterior; CuA/CuP, cubital anterior/posterior; AA, anal anterior.

(C–F) Palaeodictyoptera family indet., ISEZ PAN IF-MP-1576-326-10, Late Carboniferous, Bashkirian, Upper Silesian Coal Basin, Sosnowiec−Klimontów, Poland.

(C) Photograph of thoracic part of exuvial cuticle including wing pads: sc, scutum; scl, scutellum.

(D) Reconstruction of original exoskeleton and wing pad tracheation: msw, mesothoracic wing pad; mtw, metathoracic wing pad; ptw, prothoracic wing pad (drawn by M.P.).

(E and F) Micrographs of basal parts of prothoracic and mesothoracic wing pads. Arrows indicate developing articulary part to scutum allowing movement (white) and posterior part of wing pad fused to scutellum (red).

(G–I), *Lycodemas* cf. *adolescens*, FM PE31983, Late Carboniferous, Moscovian, Mazon Creek Lagerstätte, Illinois, USA.

(G) Photograph of wing pad.

(H) Reconstruction of wing pad including articulated joint (drawn by M.P.).

(I) Micrograph of basal part of wing pad. Arrows indicate developing articulary part to scutum allowing movement (white), posterior part of wing pad fused to scutellum (red), and wing pad axillary cord (green).

Scale bars of (A)–(D) represent 3 mm; scale bars of (E), (F), and (I) represent 1 mm; scale bars of (G) and (H) represent 5 mm.

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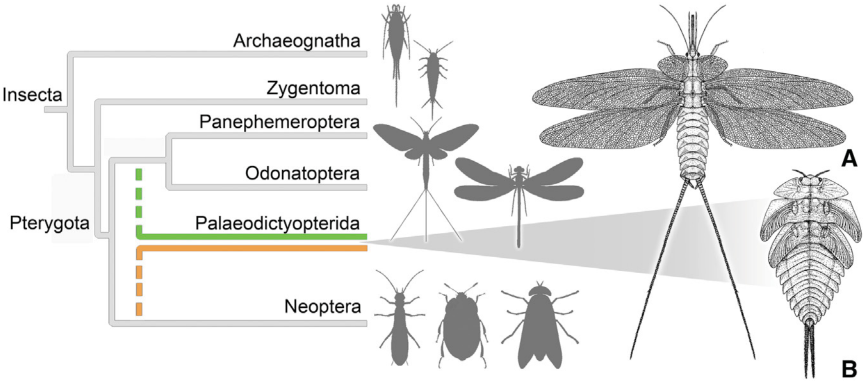

Figure 4. Two Hypotheses on the Phylogenetic Position of Palaeodictyopterida

Simplified insect phylogeny, based on a character matrix reported previously by Misof et al. [36], with the addition of extinct Palaeodictyopterida (= Palaeodictyoptera and their relatives) as sister group of Palaeoptera after Kukalová-Peck [13] (green) and as sister group of Neoptera (orange) after Sroka et al. [14].

(A) *Stenodictya lobata*, adult palaeodictyopteran male, reconstruction of habitus, Late Carboniferous, Gzhelian, Commentry, France (drawn by M.P.).

(B) *Rochdalia parkeri*, palaeodictyopteran nymph, reconstruction of habitus, Late Carboniferous, Bashkirian, Rochdale, Lancashire, UK (drawn by M.P.).

longitudinal line (mll) showing cuticular sheaths of developing wings attached to the corresponding thoracic nota (Figures 3C–3F). The triangular forewing pad and the likely tracheation of mesothoracic and metathoracic wing pads, both posterolaterally directed, are evidence for attribution to Palaeodictyoptera, which is supported by marked corrugation of the main longitudinal ridges (likely tracheae). In contrast to prominent joints of mesothoracic and metathoracic wing pads, the prothoracic wing pads seem to be weakly joined to the prothorax, presumably reflecting limited mobility.

The wing pad of *Lycodemas* cf. *adolescens* from Mazon Creek (No. FM PE31983) presumably belongs to a more mature nymphal instar since it is included within a sheath [28] (Figures 3G–3I). Earlier work omitted mention of the wing base, but examination of the fossil shows a partially preserved base with articulation and wider connection to the scutellum as in material already discussed. However, it differs from the aforementioned species in this posterior fusion markedly reduced, supporting the conclusion that this fossil is of a more mature nymphal instar.

DISCUSSION

Insect wings are unquestionably one of the most significant innovations in animal evolution, underpinning the considerable success of insects by aiding their multiple phases of diversification that today have left them the unrivaled champions of terrestrial biodiversity. Insects were the first animals to evolve powered flight and did so early in their evolution [1, 2, 29, 30]. The origin of insect wings has been one of the more contentious topics in evolutionary biology. After a century of debate, the matter today centers around two principal competing hypotheses—one positing that the wings are derived from the upper portion of the thoracic exoskeleton (notal origin) [31, 32], the other that wings are modified from an element among the appendages and lateral body wall (pleural origin; the ‘‘exite’’ theory) [13, 20, 33]. The pleural-origin hypothesis previously had defense from paleontological work, owing to the purported lack of connections to the notum during development (which we have shown to be incorrect as there were broad anterior and posterior connections in all observed nymphs). Prior genetic data also supported the pleural-origin hypothesis through the discovery of markers of crustacean appendages in developing wings of Hexapoda [34], although these could not rule out alternative origins for large swaths of other wing tissues. The opposing poles of this long debate have recently been brought closer together by the consideration of a hybrid hypothesis [2], and this view has gained support from growing evolutionary developmental evidence [3]. In this model of wing origin, tissues from both the notum and the pleuron are brought together to form a composite structure and achieve a fundamentally novel function [4–6]. The genetic mechanism responsible for the formation of an extended wing foil and some basal elements involved in stabilizing its movements are of notal derivation, while the actual articulation and associated musculature that permit proximal control during flight stem from podites of the arthropod appendage [1, 5, 35]. Combination of these different components resulted in the formation of articulated wings—the former giving the bulk of the wing, the latter the hinge. A variety of species in the extinct Palaeodictyoptera (Figure 4) and Geroptera (Odonatoptera) had wings on all three thoracic segments, although the anterior-most pair was small and perhaps not fully mobile. Subsequent increase of the mesothoracic and metathoracic segments, reduction of the prothorax, and increase in wing size allowed for better control over flight, using only four wings [16].

Although paleontological evidence has provided critical insights into the timing of origin and radiation for major insect clades [37], there are considerable gaps that have made it difficult to make direct conclusions regarding the earliest fliers and the development of their wings. Progress in phylogenomics has provided corroborating support of the timing of these events [36] but understandably has not been able to directly address the evolutionary origin of wings and their earliest anatomical arrangement.

The evaluation of available data on postembryonic development of wings in Palaeodictyoptera demonstrates three pairs of posterolaterally directed and articulated wing pads. However, the joint of the prothoracic pair was reduced by comparison to those of the mesothoracic and metathoracic segments and in relation to the smaller size of the former. Two distinct plates, a basisubcostal and large axillary plate, were present in these nymphs. The network of ridges on wing pads, which seemingly corresponds to internal tracheation, was already well developed and shows the distinctive corrugation of later developing, longitudinal veins. Notable presence of a broad, contiguous connection of the wing pads to the scutellum presumably allowed only limited divergence, and these were thus probably coopted in nymphs for thermoregulatory purposes [38] or at most limited gliding [16]. Unsurprisingly, these nymphs were likely unable to perform active flight due to the prominent notal fusion of the wing pads and because wing loading would be absurdly high [39]. The clear notal connections, overlooked or minimized in earlier studies, corroborate the dual model for insect wings origins, and this is particularly critical given that these ontogenetic forms occurred in Paleozoic insects still bearing three pairs of movable wing pads. In addition, these Paleozoic forms had well-developed articulary sclerites alongside a broad fusion of the wing pads to the scutellum. Collectively, these observations represent the first direct, paleontological support from the ontogeny of early pterygotes regarding the long-standing debate over insect wing evolution and tie nicely with modern evolutionary developmental and genomic evidence.

EXPERIMENTAL PROCEDURES

The present results were obtained by comparative morphological study using optical and digital stereomicroscopy, ESEM, 3D image modeling, and micro-computed tomography. Stereomicroscopic observations were performed using a Leica MZ 12.5. Photographs were taken with a Canon D550 digital camera, with MP-E 65 mm and EF 50 mm lenses. The original photographs were processed using Adobe Photoshop CS6, and for some images, the focus-stacking software Helicon focus Pro was used. Scanning electron micrographs of uncoated specimens were obtained using an environmental electron microscope Hitachi S-3700N at an accelerating voltage of 15 kV and a digital microscope Keyence VHX VH-Z20UR, both located at the National Museum in Prague. A micro-computed tomography dataset was acquired at the CEITEC in Brno, Czech Republic. The study of sample IF-MP 1492/365/09 was done using GE phoenix|X-ray tomography system v|tome|x. The spatial resolution of the dataset is 18 μm^3 per voxel. The 3D model of dorsal relief acquired from CT data was obtained with the help of Amira software.

Institutional abbreviations are as follows: FM, The Field Museum (Chicago); ISEZ PAN, Natural History Museum of the Institute of Systematics and Evolution of Animals PAS (Cracow); MM, Manchester Museum (Manchester); NHM, The Natural History Museum (London); YPM, Peabody Museum of Natural History Yale University (New Haven).

AUTHOR CONTRIBUTIONS

J.P. and M.P. conceived the initial idea and designed the project. E.K. and W.K. provided new fossil specimens to study. J.P., M.P., and T.H. examined material by light and digital stereomicroscopy and performed ESEM and micro-CT analyses. J.P. and M.P. produced the figures and the illustrations. J.P., M.P., A.N., T.H., E.K., W.K., and M.S.E. analyzed the data, discussed the results, and drafted and commented on the manuscript.

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Článek 7

Prokop, J., Nel, A., Engel, M. S., Pecharová, M. & Hörnschemeyer, T. 2016. New Carboniferous fossils of Spilapteridae enlighten postembryonic wing development in Palaeodictyoptera. *Systematic Entomology* 41: 178–190.

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New Carboniferous fossils of Spilapteridae enlighten postembryonic wing development in Palaeodictyoptera

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Abstract. A new palaeodictyopterid nymph *Bizarrea obscura* **gen.n.** et **sp.n.** (Spilapteridae) and a new adult specimen of *Homaloneura* cf. *dabasinskasi* Carpenter are described from the Pennsylvanian (Moscovian) ironstone nodules of Mazon Creek (IL, U.S.A.). Both taxa share enlarged prothoracic lobes (interpreted by some as winglets), heteronomous meso- and metathoracic wing pads or wings, a slender abdomen with pointed laterotergites, and a unique division of the abdominal segments by two transverse sulci. An alternative hypothesis for the placement of *Bizarrea* within Homoiopteridae is considered on the basis of its large body size and relatively short wing pads. Based on the morphology of the new material, postembryonic development of wing pads in Palaeodictyoptera (Palaeodictyopterida) is reconsidered. Detailed investigation of the abdominal segments, including examination by scanning electron microscopy, reveals the presence of subcircular, sclerotized structures partially covered at the bases of the nymphal laterotergites I–VII, ?VIII. Based on their position and shape, these structures are interpreted as abdominal spiracles, and thus a terrestrial or semiaquatic habitat for these immatures is hypothesized. Moreover, our discovery of the same, supposedly homologous structures in the enigmatic Vogesonymphidae (Permoplectoptera), from the Middle Triassic of Grès à Voltzia in France, is evidence for the parallel coexistence of ancestrally terrestrial and derived aquatic lineages of Ephemerida (Ephemeropterida) in early Mesozoic ecosystems.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:A7270D99-5B48-4EAC-AEB8-EFB8A9F55FBD.

Introduction

Palaeodictyoptera are a specialized group of Paleozoic insects that did not survive the Permian–Triassic mass extinction, yet were diverse and seemingly abundant during the Carboniferous and Permian periods (Rasnitsyn & Quicke, 2002; Grimaldi & Engel, 2005). The group is remarkable not only for its complex diversity, but also for the possession of piercing-sucking mouthparts, suggesting a herbivorous life history for the entire clade. Indeed, fluid-feeding traces of palaeodictyopterans are known from Late Carboniferous tree ferns (e.g. Labandeira & Phillips, 1996), demonstrating the convergent use of their mouthparts in comparison to modern Hemiptera. Despite a reasonable knowledge of adult palaeodictyopteran morphology, their immature stages remain poorly studied, particularly owing to the scant number of available fossils and frequently their imperfect state of preservation. Nonetheless, several exceptions are known from sphero-siderite concretions of the English Coal measures, such

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as *Idoptilus onisciformis* Wootton or *Rochdalia parkeri* Woodward, and which show fine structures (Wootton, 1972). However, the systematic placement, even to the level of family, is often debatable mainly due to progressive wing tracheation. *Idoptilus peachii* (Woodward), an early palaeodictyopteran instar, was even originally thought to be a cockroach nymph (Ross, 2010). Some of the more critical treatments of immature Palaeodictyoptera include the work by Sinitshenkova (1979), who described a series of fossils as the nymphal, subimaginal and adult stages of putatively a single species, *Paimbia fenestrata* Sinitshenkova (Tchirkovaeidae), found in the Late Carboniferous (Gzelian – Kasimovian) of Tunguska-Chunya, Central Siberia, while Kukalová-Peck & Peck (1976) described the mesothoracic wing pads of a young nymph and of a subimago from the Lower Permian (Artinskian) of Obora, Czech Republic. More recently, Staniczek *et al.* (2014) provided a revision of the controversial hexapod fossil *Carbotriplura kukalovae* Kluge, originally considered as the nymph of a giant palaeopteran pterygote insect, and suggested it as sister to Pterygota, contrary to Kukalová-Peck (1985) and Kluge (1996). Engel *et al.* (2013) broadly reviewed current evidence regarding molecular, genetic, developmental and palaeontological evidence for insect wing evolution and origins, and Haug *et al.* (2014) have provided a similar summary focusing on the development of Palaeozoic insects in general. All of these works have highlighted the importance of more fully understanding the Palaeozoic diversity of insects, in particular the morphology, development and life histories of their immature stages.

Traditionally, the superorder Palaeodictyopterida comprised Palaeodictyoptera together with other groups that share certain modifications of the mouthparts into a rostrum with five long stylets, this constituting a putative synapomorphy for the clade; however, many palaeodictyopterid fossils do not preserve the head and so associations are usually by wing venational attributes. The clade is currently treated as an extinct, herbivorous lineage of Palaeoptera closely related to Ephemeropterida (Odonatoptera and Ephemerida), together sharing the incapability of folding the wings over the abdomen during rest. Monophyly of Palaeoptera has been controversial (e.g. Grimaldi & Engel, 2005; Trautwein *et al.*, 2012; Thomas *et al.*, 2013), and recently Sroka *et al.* (2014) proposed the placement of Palaeodictyopterida as sister to Neoptera, resurrecting the former hypothesis of Willmann (2004). Clearly, the placement of the various palaeopteran clades remains contentious and further data are needed, including extensive exploration for immatures from Palaeozoic deposits.

The Mazon Creek locality, situated in northeastern Illinois, USA, is one of the best-known Carboniferous conservation sedimentary deposits (*Lagerstätte)* in North America, preserving animal and plant fossils in ironstone concretions customarily called nodules. The fossil insect record of corresponding strata such as the Francis Creek Shales Member includes 140 described species and 106 genera in 36 families (according to the EDNA Fossil Insect Database at http://edna.palass-hosting.org/). Palaeodictyoptera from the Mazon Creek locality comprise six families (Carpenter, 1997) represented by fossils of both adult and immature stages, making this a particularly critical deposit for understanding Palaeozoic insect evolution. Size disparity in this group is remarkable, ranging from the smallest species, such as *Eubleptus danielsi* Handlirsch (Eubleptidae), with a wingspan of just about 30 mm, to giants such as *Mazonopterum wolfforum* Kukalová-Peck & Richardson (Homoiopteridae), with wingspans up to 368 mm (Handlirsch, 1906; Carpenter, 1965; Kukalová-Peck & Richardson, 1983). Homoiopteridae are represented by five genera, including a putative homoiopterid nymph, *Adolarryia bairdi* Kukalová-Peck & Richardson, and the subimago *Larryia ostenbergi* Kukalová-Peck & Richardson, the latter bearing a prominent cavity on the anterior forewing margin (Kukalová-Peck & Richardson, 1983). The family Lycocercidae is known by *Notorachis wolfforum* Carpenter & Richardson, with prominent spines on the prothorax and the nymph *Lycodemas adolescens* Carpenter & Richardson, which possesses a venation that is quite similar to *Lycocercus* (Carpenter & Richardson, 1971). Distinctions between Homoiopteridae and Lycocercidae can at times be difficult to discern, and both families should be revised and more fully circumscribed (e.g. Beckemeyer & Engel, 2011). The largest palaeodictyopteran group, the family Spilapteridae, is represented in Mazon Creek by three genera, *Homaloneura* Brongniart, *Spilaptera* Brongniart and *Mcluckiepteron* Richardson. A final species, *Neofouquea suzannae* Carpenter & Richardson, is attributed to Fouqueidae.

Here we provide a description of an additional specimen tentatively assigned to *Homaloneura* cf. *dabasinskasi* Carpenter, that partially preserved the abdomen, thereby providing information on these structures for the genus, and a new palaeodictyopteran nymph, *Bizarrea obscura* **gen.n.** et **sp.n.** Both are from the Pennsylvanian of Mazon Creek. Our comparative study of both taxa suggests their likely placement in Spilapteridae, mainly based on the external morphology of the abdomen. Moreover, an alternative placement within Homoiopteridae is discussed based on the size of the wing pads in comparison to body length, but is considered less likely. Of importance, the presence of relatively short wing pads in a presumably late instar precludes the gradual development of wings in Spilapteridae, and possibly in all Palaeodictyoptera. Accordingly, in contrast to previous hypotheses (e.g. Sharov, 1973; Sharov & Sinichenkova, 1977; Kukalová-Peck, 1991, 1997, 2008), we suspect allometric growth of wings during postembryonic development in Palaeodictyopterida, and quite intuitively as one would expect in a mode that is at least somewhat similar to extant, nonholometabolous Pterygota.

Material and methods

Two specimens preserved in sphero-siderite nodules from the Carbondale Formation (Francis Creek Shale Member) of Illinois, U.S.A., were examined. Both compression fossils partially bear three-dimensional relief of the original insect habitus, including their corrugated wing venation. The material was originally stored in the private collection of Ms Gertrude Hannen, but is now housed in the Field Museum of Natural History (Paleontology) in Chicago, U.S.A.

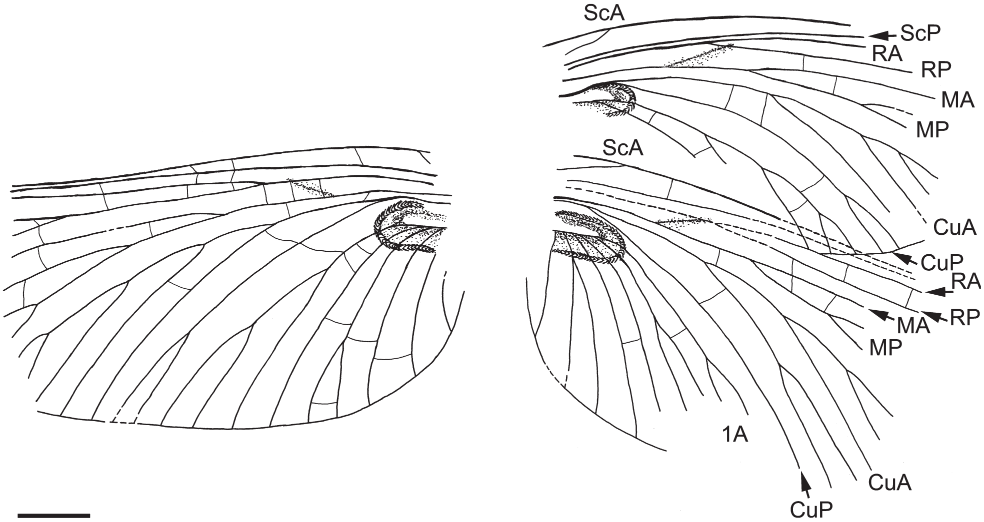

Fig. 1. *Homaloneura* cf. *dabasinskasi* Carpenter, 1964, specimen no. PE 11268, the Field Museum of Natural History (Paleontology), Chicago, U.S.A.; drawing of wing venation (scale bar = 5 mm). ScA/ScP, subcostal anterior/posterior; RA/RP, radial anterior/posterior; MA/MP, medial anterior/posterior; CuA/CuP, cubital anterior/posterior; 1A, first anal vein.

The fossil specimens were observed with Olympus SZX-9 and Nikon 645 stereomicroscopes in a dry state. Line drawings of the pattern of wing venation and habitus were made directly through a stereomicroscope Leica MZ 12.5 with the aid of a camera lucida. Photographs were taken with a Canon D550 digital camera, with MP-E 65 mm and EF 50 mm lenses. The original photographs were processed using Adobe PHOTOSHOP CS6, and for some images the focus-stacking software HELICON FOCUS PRO was used. Scanning electron micrographs of the holotype were taken by an environmental electron microscope Hitachi S-3700N in the Department of Palaeontology of the National Museum in Prague.

Micro-computed tomography (micro-CT) datasets were acquired for both specimens at the CEITEC - Central European Institute of Technology in Brno, Czech Republic. The study of both samples was done using GE phoenix|x-ray tomography system v|tome|x. The spatial resolution of the dataset for the nymph (PE 11269) is $(55\,\mu m)^3$, for the adult (PE 11268) it is $(50\,\mu m)^3$ per voxel. For acquisition of the X-ray data for the adult specimen, plates and counter-plates were mounted side by side on the same specimen holder with a few millimetres of empty space between them. Thus, only one scan was necessary for acquiring data for both plates, while subsequently allowing for easy separation of the data for each plate with the help of AMIRA software (FEI, Hillsboro, OR, U.S.A.).

The wing venation nomenclature follows Kukalová-Peck (1991), with the following symbols used for the wing veins (symbols in capitals denote the longitudinal veins): ScA/ScP, subcostal anterior/posterior; RA/RP, radial anterior/posterior; MA/MP, medial anterior/posterior; CuA/CuP, cubital anterior/posterior; 1A/2A, first/second anal vein.

Systematics

Order Palaeodictyoptera Goldenberg, 1877: 8.
Superfamily Spilapteroidea Brongniart, 1893: 334.
Family Spilapteridae Brongniart, 1893: 334

Type genus. Spilaptera Brongniart, 1885: 83.

List of included genera (after Li *et al.*, 2013): *Abaptilon* Zalessky; *Baeoneura* Sinitshenkova in Sharov & Sinitshenkova; *Becquerelia* Brongniart; *Delitzschala* Brauckmann & Schneider; *Dunbaria* Tillyard in Dunbar & Tillyard; *Epitethe* Handlirsch; *Homaloneura* Brongniart; *Lamproptilia* Brongniart; *Mcluckiepteron* Richardson; *Neuburgia* Martynov; *Palaeoptilus* Brongniart; *Paradunbaria* Sharov & Sinitshenkova; *Permiakovia* Martynov; *Spilaptera* Brongniart; *Spiloptilus* Handlirsch; *Sinodunbaria* Li *et al.*; *Tectoptilus* Kukalová; and *Vorkutoneura* Sinitshenkova in Sharov & Sinitshenkova.

Genus Homaloneura Brongniart, 1885

Type species. Homaloneura elegans Brongniart, 1885.

Homaloneura cf. dabasinskasi Carpenter, 1964

Figs 1, 2; Figure S1

Description. Specimen PE 11268 (part and counterpart), preserving the remains of an adult animal, estimated body length about 50 mm, hypognathous head 2.5 mm long with prominent and trapezoidal clypeus, thoracic segments fragmentary and partly distorted, prothorax short with medial keel present close

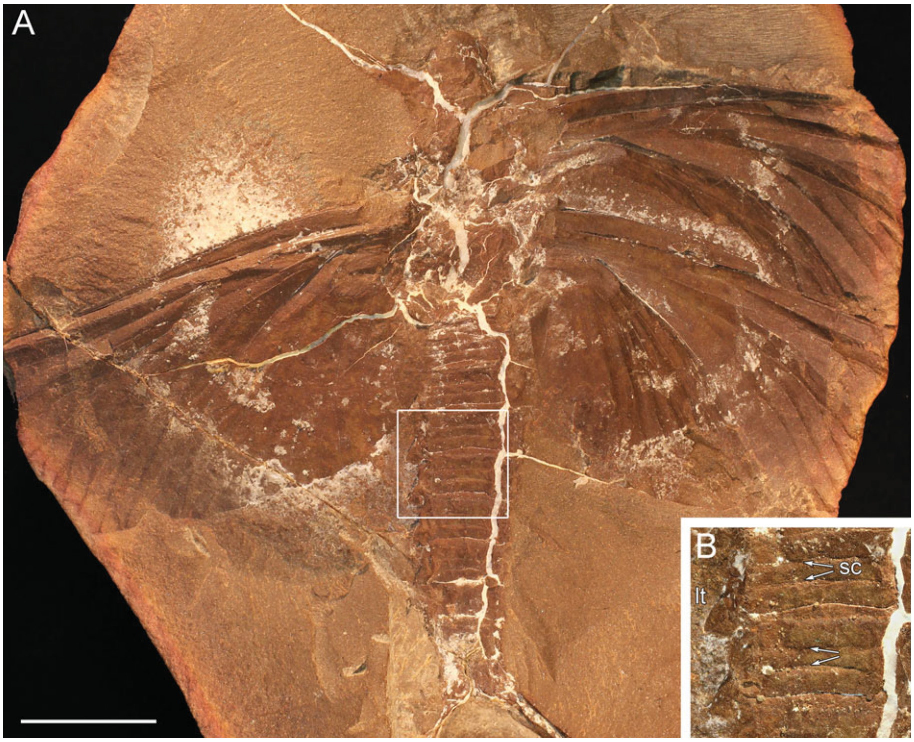

Fig. 2. *Homaloneura* cf. *dabasinskasi* Carpenter, 1964, specimen no. PE 11268, the Field Museum of Natural History (Paleontology), Chicago, U.S.A. (A) Photograph of habitus; (B) detail of abdominal segments III and IV with indication of transverse sulci (sc) and cordate laterotergites (lt) (scale bar = 10 mm).

to anterior margin; preserved legs with stout coxae; right mesothoracic and a pair of metathoracic wings well preserved in basal half, corresponding apical parts not preserved; wing venation with strongly corrugated longitudinal veins connected by simple, thin transverse crossveins; wing membranes without traces of colouration.

Forewing broadest at about midpoint, width at level of midpoint 15.4 mm, costal margin slightly curved; convex ScA reaching costal margin about 4.1 mm from wing base, nearly straight ScP running parallel to convex RA; basally broad area between ScP and costal margin becomes narrow at about midpoint; veins RA and RP basally simply connected, RA nearly straight, RP diverges from RA about 13.4 mm from wing base; division of M into MA and MP close to separation of RA and RP but 2.6 mm more distal; convex MA without basal branching; concave MP deeply branched 7.5 mm behind division of MA and MP; convex cuticular ridge in form of oblique crossvein present between M and RP 10.0 mm from wing base; division of CuA and CuP close to wing base; CuA pectinate with four branches, concave CuP terminally with two branches; two simple crossveins in area between CuA and CuP; anal area basally connected by prominent anal brace; first anal vein distinctly pectinate ending with four branches.

Hindwing venation pattern similar to forewing with slightly concave costal margin and distinctly broader anal area; ScA reaching costal margin about 3.0 mm from wing base; ScP nearly straight, basally broad area between ScP and costal margin becoming distinctly narrow by midpoint; RP diverging from RA about 14.0 mm from wing base, RP distally branched about 13.1 mm from division of RA and RP, division of MA and MP 2.4 mm distal from separation of RA and RP; simple MA well delimited from RP; MP with at least three terminal branches, first branch 5.9 mm from division of MA and MP; convex cuticular ridge in form of oblique crossvein present between M and R 8.8 mm from wing base (on M); division of CuA and CuP 5.2 mm from wing base; CuA pectinate, ending with four to five branches, first branch simple or bifurcate, concave CuP terminally with one to two branches; anal area formed by six main veins, first anal vein pectinate and reaching posterior wing margin with two or three terminal offshoots, anal veins basally connected by strong anal brace; relatively few simple crossveins connecting anal veins.

Abdomen incompletely preserved with eight distinct basal segments, width of first tergite 5.7 mm, all secondarily subdivided into three or four parts of more or less equal size by transverse, parallel furrows (sulci). Abdominal segments II–VII bear pointed, cordate laterotergites and oriented backwards and downwards.

Dimensions. Estimated body length 50 mm; prothorax length 4.1 mm, width 4.7 mm; preserved forewing length 29.0 mm, width at widest part 15.6 mm, preserved hindwing length right 29 mm, left 34 mm (estimated total length about 60 mm), width at widest part 18.7 mm (16.7 mm midwing), estimated wingspan about 110–120 mm; incomplete abdomen length 28.2 mm, width 5.5 mm, estimated total body length about 50.3 mm.

Material. Specimen PE 11268 (part and counterpart), housed in the Field Museum of Natural History (Paleontology), Chicago, U.S.A.; originally from the collection of Ms Gertrude Hannen. Adult with fragmentary head, prothorax with fragmentary prothoracic leg, poorly preserved meso- and metathorax bearing outstretched wings, and partly preserved abdomen.

Locality and strata. Francis Creek Shale Member, Carbondale Formation, Braidwood, Illinois, USA; Late Carboniferous, Pennsylvanian, Moscovian (Westphalian C/D).

Discussion. This fossil belongs to the palaeodictyopteran family Spilapteridae as evidenced by the combination of the following characters present in the wing venation: (i) anterior wing margin slightly concave medially; (ii) MP with two or more terminal branches; (iii) CuA with four or more terminal branches; (iv) hindwings broader than forewings and with a more developed anal area. This family is known by 19 genera from the Late Carboniferous and Permian deposits of Euramerica, Siberia and northern China (Carpenter, 1992; Sinitshenkova, 2002; Li *et al.*, 2013).

The fossil described herein is attributed to *Homaloneura* due to the presence of the following characters: anterior wing margin slightly concave, area between RA and RP with only straight simple crossveins, M free of RP, MP with at least three terminal branches, CuA pectinate and ending with four to five terminal branches, and prominent anal brace in the form of a cuticular ridge extending to RP/R. However, this genus is strikingly similar to *Spilaptera*, differing only by characters such as the more concave anterior wing margin, the area between RA and RP having sigmoidal crossveins, and a deeply branched CuP; as discussed by Kukalová (1969). These diagnostic characters are variable among the known species and the possibility that the two genera should be considered synonyms cannot be excluded. In addition, we note the plasticity of certain characters like the reinforced anal brace extending to R, a feature putatively typical of *Homaloneura*, occurring in some species of *Spilaptera*, e.g. *Spilaptera splendens* Prokop *et al.* (Prokop *et al.*, 2014).

The genus *Homaloneura* is known from 11 species from the Late Carboniferous (Moscovian) of Commentry in France (*H. elegans* Brongniart, *H. bonnieri* Brongniart, *H. punctata* Brongniart, *H. joannae* Brongniart, *H. ornata* Brongniart, *H. lehmani* Kukalová, *H. bucklandi* Brongniart), from the Namurian of Hagen-Vorhalle and from the Moscovian of Piesberg, Germany (*H. berenice* Brauckmann & Groning, *H. killiani* Brauckmann, Herd & Leipner, *H. ligeia* Brauckmann), as well as from the Moscovian of Mazon Creek (*H. dabasinskasi* Carpenter). The pattern of venation of the fossil described here fits well with *H. dabasinskasi* Carpenter, known from the same locality (holotype and an additional specimen with incomplete preservation) (Carpenter, 1964; Carpenter & Richardson, 1971). The holotype of *H. dabasinskasi* preserves only as isolated forewing and hindwing, with the original pattern of colouration, but according to the drawings of Carpenter (1964), most of the basal areas of the wings are missing. Based on our re-examination of the original photograph (Carpenter, 1964: plate 15), the anal area is probably more completely preserved than indicated on the corresponding drawing (Carpenter, 1964: plate 16). The second specimen assigned to this species is poorly preserved, showing only parts of the thoracic segments and the base of the forewing with a clearly visible V-shaped anal brace, which is not indicated in the corresponding drawing.

We provide a description of the present specimen so as to expand our knowledge of this species, particularly in the documentation of the basal wing structures and form of the abdomen. Moreover, the holotype is stored in a private collection and generally not accessible, while the new specimen is housed in an institutional collection and is readily available to researchers.

Genus Bizarrea **gen.n.**

http://zoobank.org/urn:lsid:zoobank.org:act:5D945AEA-5B06-4812-9294-948FEDA7C012

Type species. Bizarrea obscura **sp.n.**, here designated.

Etymology. Named after the strange habitus of the nymph (‘bizarre’ in English) with prominent, apically pointed abdominal laterotergites; the gender of the name is feminine.

Bizarrea obscura **sp.n.**

http://zoobank.org/urn:lsid:zoobank.org:act:6C9208B3-C024-4A0A-838D-87164C283016

Figs 3–6, Figure S2

Diagnosis. Based on an immature stage with rostrate mouthparts composed of elongate stylets, expanded prothoracic lobes (winglets), small meso- and metathoracic wing pads; metathoracic wing pads slightly shorter and basally broader in comparison to those of the mesothorax; comparatively slender abdomen, consisting of ten segments bearing distally-pointed laterotergites; abdominal segments I–VII with prominent, transverse sulci subdividing them into three, nearly equal, parts.

Etymology. The specific epithet refers to the state of preservation with some barely visible morphological structures.

Description. Large nymph with prominent rostrate mouthparts, slightly expanded prothoracic lobes, two pairs of short wing pads with corrugated venation directed obliquely along body; abdomen with ten abdominal segments bearing distally pointed laterotergites.

Head poorly preserved and bearing relatively long, filiform antennae with an enlarged scape, pedicel not discernible, and several thin antennomeres; faintly preserved rostrate mouthparts with basally stout, elongate stylets (disarticulated).

Fig. 3. *Bizarrea obscura* **gen.n.** et **sp.n.**, holotype specimen no. PE 11269, the Field Museum of Natural History (Paleontology), Chicago, U.S.A. (A) Photograph of habitus, prothoracic lobes (?winglets) (pw), pointed abdominal laterotergites (lt), cerci (ce); (B) detail of head with mouthparts in the form of stylets (st), antennae (an); (C) detail of abdominal segments I–III with indication of transverse sulci (sc), hidden subcircular structures close to the anterior margin of the corresponding laterotergite (cs) (scale bar = 10 mm).

Prothorax poorly preserved, bearing a pair of small fragmentary paranotal lobes without distinct pattern of tracheation (venation), and markedly shorter than wing pads, meso- and metathoracic wing pads with partly preserved venation by progressive tracheation, partial veinal corrugation visible; metathoracic wing pads slightly shorter and basally broader in comparison to those of mesothorax.

Abdomen slender, consisting of ten distinct segments tapering towards apex, each segment bears a pair of basally broad laterotergites with pointed apices; each laterotergite distinctly elevated relative to corresponding abdominal segment, connection between abdominal tergite and corresponding laterotergite rigid; abdominal segments I–VII subdivided into three nearly equal parts and separated by prominent transverse sulci; last three segments poorly preserved in these parts; subcircular or oval structure located close to anterobasal part of each laterotergite (I–VII, ?VIII), faintly connected by trachea(?) emerging between two adjacent abdominal segments (see Fig. 5); developing ovipositor situated beneath abdominal segments VIII–X with two discernible, elongate valvulae (apparently the anterior pair) reaching about apex of segment X (see Fig. 6) (much as in extant Odonatoptera, the valvulae of the ovipositor, albeit nonfunctional, appear in late-instar nymphs and those here are in an identical, medial position and do not exceed beyond sternum X;

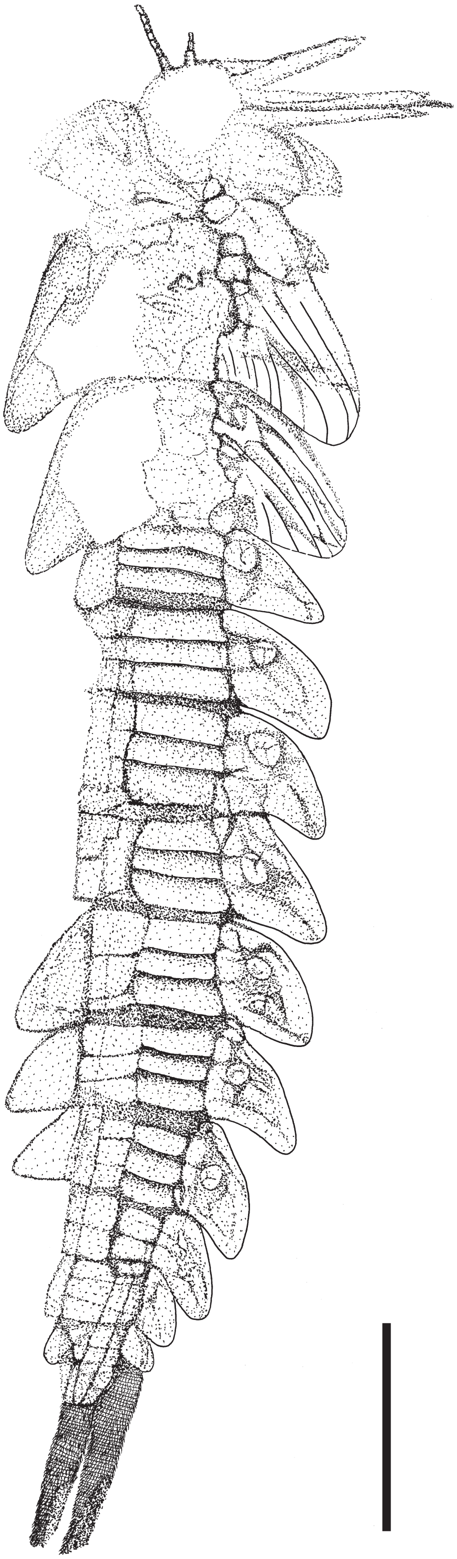

Fig. 4. *Bizarrea obscura* **gen.n.** et **sp.n.**, holotype specimen no. PE 11269, the Field Museum of Natural History (Paleontology), Chicago, U.S.A. Line drawing reconstruction of habitus (scale bar = 10 mm).

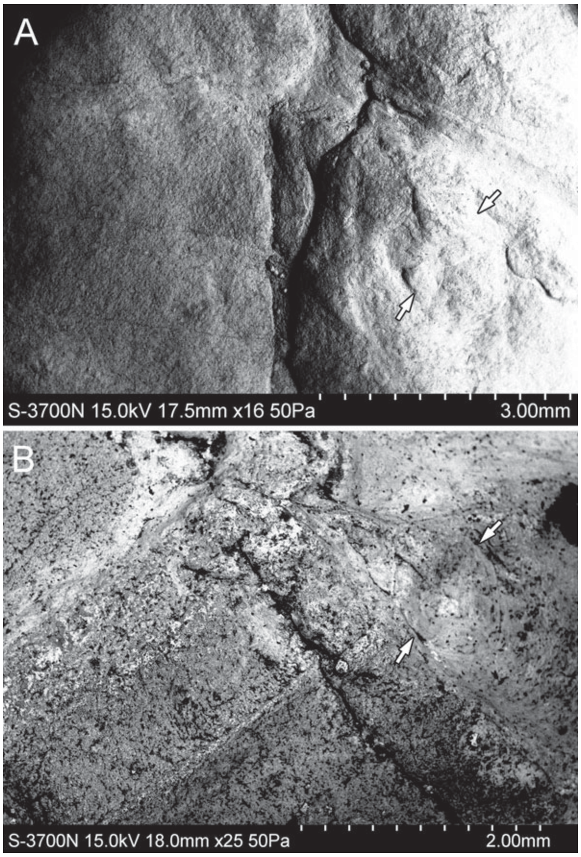

Fig. 5. *Bizarrea obscura* **gen.n.** et **sp.n.**, holotype specimen no. PE 11269, the Field Museum of Natural History (Paleontology), Chicago, U.S.A. (A) Micrograph of abdominal segment III with detail of laterotergite base; (B) micrograph of abdominal segment V with detail of laterotergite base. Arrows indicate hidden subcircular structures close to the anterior margin of the corresponding laterotergite.

e.g. Matsuda, 1976); terminal abdominal segment bearing a pair of stout multisegmented cerci covered with dense setation.

Dimensions. PE 11269: Body length 64.8 mm; prothorax length 4.4 mm, width 3.8 mm; mesothoracic wing pad length 10.3 mm, metathoracic wing pad length 10.0 mm; abdomen length 44.5 mm, width 5.6 mm.

Material. Holotype PE 11269 (part) housed in Field Museum of Natural History (Paleontology), Chicago, U.S.A.; originally in the collection of Ms Gertrude Hannen. Nymph with rather poor state of preservation, fragmentarily preserved head, prothorax with extended prothoracic lobes (?winglets), meso- and metathorax bearing small immature winglets (wing pads), abdomen with ten segments distinctly laterally extended, fragmentarily preserved ovipositor and terminal abdominal segment with a pair of stout cerci.

Locality and strata. Francis Creek Shale Member, Carbondale Formation, Will-Kankakee-Grundy Co., IL, USA; Mazon

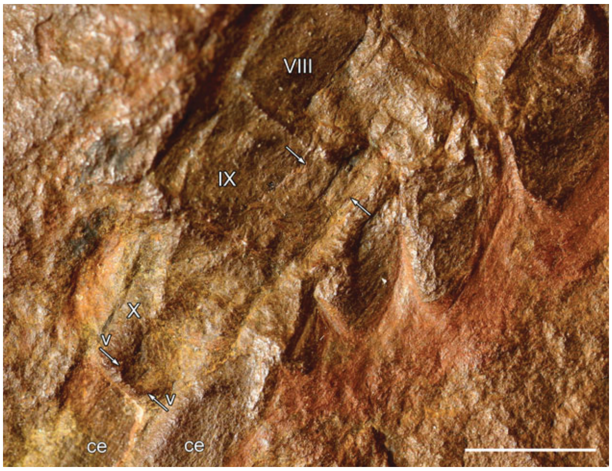

Fig. 6. *Bizarrea obscura* **gen.n.** et **sp.n.**, holotype specimen no. PE 11269, the Field Museum of Natural History (Paleontology), Chicago, U.S.A. Detail photograph of abdominal segments VII–X with indication of fragmentarily preserved ovipositor valves, arrows showing elongate valvulae (v), and cerci (ce) (scale bar = 3 mm).

Creek area; Upper Carboniferous, Pennsylvanian, Westphalian C-D (Moscovian).

Discussion. Bizarrea **gen.n.** exhibit traits for placement within Palaeodictyoptera, most notably the rostrate mouthparts with long stylets (autapomorphy), prominent prothoracic lobes known for many members of this group, heteronomous articulated meso- and metathoracic wing pads with corrugated venation, abdominal segments with prominent elevated and apically pointed laterotergites, and terminal abdominal segment bearing a pair of stout multisegmented cerci covered with dense setation. The preservation of the rostrum in immature Palaeodictyopterida is extremely rare, so far known only in the nymph of the megasecopteran *Mischoptera douglassi* Carpenter & Richardson and *Paimbia fenestrata* Sinitshenkova (Carpenter & Richardson, 1968; Sinitshenkova, 1979). Unfortunately, the poor state of preservation does not allow for a more precise reconstruction of the progressive wing tracheation, which could partly indicate the future pattern of venation, and hence the ambiguity in familial attribution. Nevertheless, based on the relative sizes of the fore- and hindwings, the slender abdomen, and particularly the unique subdivision of abdominal segments I–VII by two transverse sulci (see Fig. 3C), we conclude that the nymph belongs to the family Spilapteridae. The combination of basally broader metathoracic wings and the marked transverse subdivision of abdominal segments by two sulci is unknown in other palaeodictyopterans and therefore could be considered an autapomorphy of Spilapteridae, given that it is also present in other species, such as *Homaloneura bonnieri* Brongniart (Fig. 7). However, the apomorphic state of these characters is somewhat ambiguous because the majority of fossils assigned to Spilapteridae, and even most palaeodictyopterans, are fragmentarily preserved and lacking the abdomen. Therefore, we prefer the placement of *Bizarrea* in Spilapteridae. This family is the largest of the Palaeodictyoptera, comprising 19 genera, but until now without fossils from immature stages (Li *et al.*, 2013).

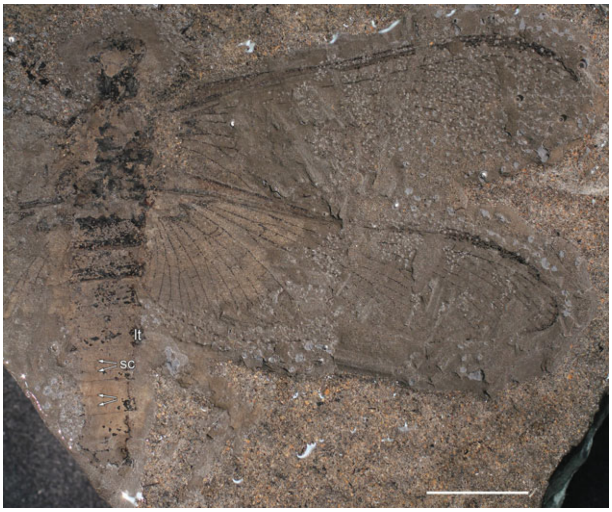

Fig. 7. *Homaloneura bonnieri* Brongniart, 1885, R51302, Muséum national d'Histoire naturelle, Paris, France; photograph of holotype (horizontally flipped) with abdominal segments bearing transverse sulci (sc) and pointed laterotergites (lt) (scale bar = 10 mm).

With representatives of three genera, *Homaloneura*, *Spilaptera*, and *Mcluckiepteron* Richardson (Carpenter, 1964; Carpenter & Richardson, 1971), the family Spilapteridae is a significant group of palaeodictyopterans in the Mazon Creek locality. However, the latter genus is based only on the poorly preserved type species, *M. luciae* Richardson, and therefore its family assignment has been questioned (Carpenter, 1992).

Surprisingly, all species assigned to the aforementioned genera reach maximum body lengths of about 55 mm as adults. This does not correspond well with the early nymphal instars, which reach a total body length of about 70 mm while having quite small wing pads. Even if we admit significant allometry between body length and wing pads during ontogenesis, such a discrepancy might indicate the presence of an unknown member of Spilapteridae closely related to *Homaloneura*, or of another palaeodictyopteran family. Nonetheless, nymphs of Aeschnidiidae (Odonata) found in the Early Cretaceous of China have bodies larger than the corresponding adults (Fleck & Nel, 2003). Thus, the large nymph of *Bizarrea* **gen.n.** could also correspond to a smaller (at least slightly) adult.

It should be noted that Sharov (1973), Sinitshenkova (1979), and Kukalová-Peck (1991, 1997, 2008) supposed a gradual growth of wing pads during postembryonic development, with numerous nymphal and subimaginal instars for Palaeodictyoptera, which would be unique among basal insects. Thus, relatively small winglets would correspond to early nymphal instars and in contrast to the development known from extant members of Odonata and Ephemeroptera, where growth of the wings takes place within considerably small wing pads until the last naiad stages. However, the evidence for such a general statement concerning the gradual development of wings during successive ontogenetic stages of Paleozoic insects is limited as we cannot determine with confidence the precise stage of individual nymphs. The patchy fossil record documents only a few nymphal instars of Palaeodictyoptera with small wing pads in oblique or even perpendicular position to the body axis (e.g. Wootton, 1972; Kukalová-Peck & Peck, 1976; Sinitshenkova, 1979; Prokop *et al.*, 2013; Haug *et al.*, 2014). Assuming the correct placement of *Bizarrea* here, it contradicts the assumption of gradual growth of winglets during postembryonic development and thus seems to be rather similar to the mode of wing growth of extant palaeopterous insects.

Alternatively, it might be assumed that *B. obscura* belongs to the family Homoiopteridae, which includes significantly larger species. Considering the rather large size of the nymph, we could suspect a rather gradual growth during postembryonic development and estimate the final adult size to be well beyond the 70 mm of the preserved nymph, probably reaching values of more than 100 mm, and thereby fitting with members of Homoiopteridae, already known as the largest palaeodictyopterans. This family is present in the Mazon Creek fauna, and from the isolated nymph, *Adolarryia bairdi* Kukalová-Peck & Richardson. This specimen has five transverse sulci on the abdominal tergites and might represent a subadult of *Larryia osterbergi* Kukalová-Peck & Richardson (Kukalová-Peck & Richardson, 1983). Nevertheless, both latter taxa are rather fragmentarily preserved and only tentatively attributed to Homoiopteridae, primarily based on their large size and pattern of hardly traceable venation. In addition, the mesothoracic wing pad of *A. bairdi* is markedly elongate and the abdomen, even though poorly preserved, appears to be quite compact and massive with only small laterotergites, quite unlike that in the present specimen (see Kukalová-Peck, 1983: figs 19–22). Therefore, *B. obscura* most probably represents a new spilapterid species, and also provides strong evidence for a postembryonic development that was similar to members of recent palaeopterous lineages, and avoids purporting unique, *ad hoc* developmental patterns for Paleozoic insects in contrast to their modern counterparts.

Staniczek *et al.* (2014: 629–630) provided a photograph of the type specimen of *B. obscura* and briefly noted its similarity to *C. kukalovae* Kluge, particularly in the presence of marked, lateral abdominal extensions. Our observations confirm the presence of movable thoracic winglets with tracheation, as was assumed by Staniczek *et al.* (2014) from the photograph, but the abdominal laterotergites are in fact rigid paraterga lacking any traces of venation (Fig. 3C). Moreover, there seem to be structures located beneath these laterotergites that appear as subcircular or oval imprints next to the anterior part of the laterotergite base (Fig. 5A, B). These structures could be hypothesized as corresponding to abdominal tracheal gills, as are present in mayfly naiads (Ephemeroptera) or extinct members of Cretaceous *Mickoleitia* sp. (Staniczek *et al.*, 2011). However, such a conclusion would be entirely without justification as there is not even a single filament emerging from these subcircular structures, and they could not function as tracheal gills. Therefore, we conclude that these structures correspond to comparatively large spiracles lying in the pleura beneath the laterotergites, analogous to many modern terrestrial or semiaquatic insects. If spiracles, then their presence would indicate that the nymph was terrestrial (or largely terrestrial with semiaquatic phases), as the majority of branchiopneustic nymphs lack these structures. If, however, it is a late instar, the spiracles might also be those of the adult, which are already formed beneath the cuticle of the nymph.

Surprisingly, the presence of such prominent, apically pointed, and strongly sclerotized abdominal laterotergites with subcircular or oval structures lying beneath each laterotergite are known from nymphs of *Vogesonympha ludovici* Sinitshenkova & Papier (Vogesonymphidae) from the early Middle Triassic (Upper Buntsandstein) of the Grès à Voltzia Formation in Vosges, eastern France (Sinitshenkova *et al.*, 2005). The Vogesonymphidae were first considered as Pterygota *incertae sedis* and later transferred to Permoplectoptera (Protereismatina *auctorum*) (Ephemerida) together with Protereismatidae, Misthodotidae and Tintorinidae, mainly on the basis of a segmented tarsus and a doubled pretarsal claw, and the presence of a paracercus along with the cerci (Sinitshenkova, 2013). Due to marked similarities in abdominal morphology between *Vogesonympha* and *Bizarrea*, possibly reflecting the same environmental adaptation, we suspect that these are likely homologous. However, *Vogesonympha* differs from *Bizarrea* by the presence of pointed laterotergites only on segments I–VII (see Fig. 8A). Sinitshenkova *et al.* (2005) cautiously speculated about the function of these prominent laterotergites, suggesting they might be covers for the protection of delicate gills, which were otherwise not preserved (and probably never present). However, the vogesonymphids differ from *Bizarrea* by the presence of a paracercus (terminal filament), indicative of Ephemerida. A further two genera and species, namely *Khungtukunia sibirica* Sinitshenkova from the Permian–Triassic deposits of the Tunguska Basin in Siberia and *Palegonympha triassica* Sinitshenkova and Aristov from the Middle Triassic of the Pałęgi area in Poland, were also assigned to Vogesonymphidae, but they seem to be too poorly preserved for such a comparison (Sinitshenkova, 2013; Sinitshenkova *et al.*, 2015). In fact, these observations strongly suggest that these homologous structures, as interpreted herein as spiracles, thus represent adaptations for a similar lifestyle in Vogesonymphidae and also as the first evidence of the co-occurrence of aquatic and probably terrestrial nymphs of Ephemerida in the Grès à Voltzia locality (see Fig. 8B). Note that the semi-terrestrial mode of life is known from modern petal-tail dragonflies (Petaluridae), whose late-instar nymphs are amphibiotic, constructing their burrows in wetlands and bogs above the water level and waiting in close proximity to catch their prey.

We concur with the notion of progressive tracheation and marked corrugation of veins, as well as the articulation of nymphal wing pads as being present in *Bizarrea*. In contrast to Staniczek *et al.* (2014), we do not believe there is a similarity of the thoracic extensions between *B. obscura* and *C. kukalovae*. In fact, they are quite different, especially concerning the venation and progressive tracheation, as well as in the form of articulation that permits movement.

Those other immature palaeodictyopterans, *R. parkeri* Woodward and *I. denisciformis* Wootton, both known from the

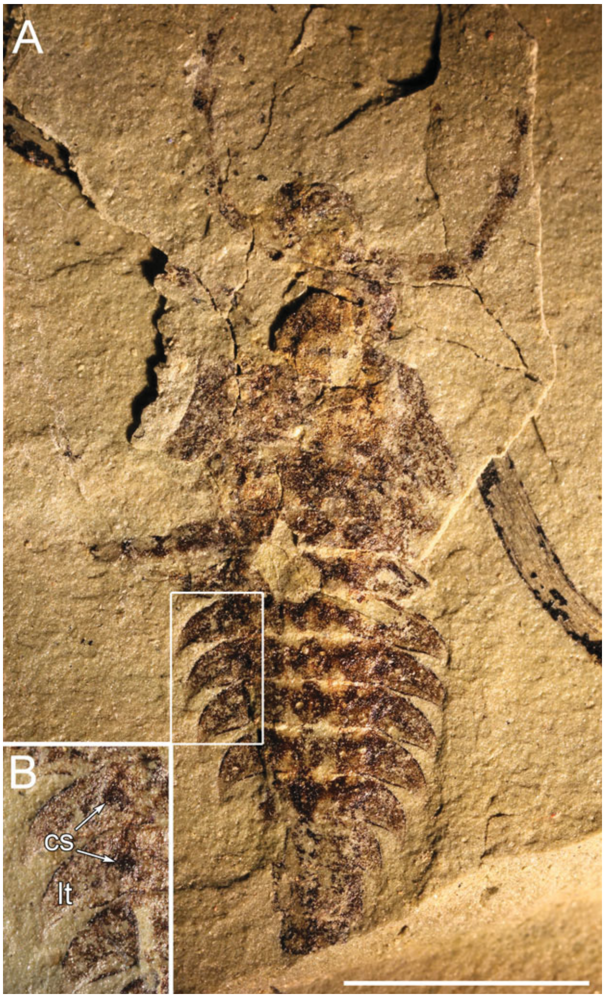

Fig. 8. *Vogesonympha ludovici* Sinitshenkova & Papier, 2005 (Vogesonymphidae), holotype no. 8036, Grauvogel-Stamm private collection, Ringendorf near Strasbourg, France. (A) Photograph of habitus; (B) detail of abdominal segments II–IV with pointed laterotergites (lt) and hidden subcircular structures (cs) (scale bar = 10 mm).

Langsettian of the English Coal Measures, show a distinctly different, onisciform habitus, with much more extensively developed abdominal laterotergites, and quite unlike those in *Bizarrea* (Wootton, 1972). These taxa were only tentatively assigned to the families Eugereonidae or Breyeriidae for *Idoptilus*, and Dictyoneuridae for *Rochdalia*.

Carpenter & Richardson (1971) reported on the nymph *L. adolescens* Carpenter & Richardson from Mazon Creek, a species that bears a pattern of venation comparable to *Lycocercus*, and its placement in Lycocercidae seems accurate. *Lycodemas* mainly differs from *Bizarrea* by lacking prominent prothoracic lobes, by its slender homonomous wing pads, and the strongly reduced abdominal laterotergites.

To definitively resolve the life history of *Bizarrea* is challenging, mainly owing to the poor state of preservation. Nevertheless, subcircular or oval, well-sclerotized structures without filaments (spiracles), such as those seen in the basal anterior part of the laterotergites of the fossil, are present in many terrestrial or semiaquatic insects, e.g. in extant moss bugs (Coleorrhyncha: Peloridiidae) or in the larvae of some carrion beetles and fireflies (Coleoptera: Silphidae, Lampyridae). In these species, the laterotergites have a protective function, and we might conclude the same was true for *Bizarrea*. The need for continued exploration for immature Paleozoic insects is only heightened by the tantalizing evidence before us.

Growth of wings during postembryonic development in Palaeodictyoptera

The record of palaeodictyopteran immatures is limited due to their scarcity and often fragmentary preservation. Our knowledge of postembryonic development of palaeodictyopterans is mainly based on the works of Wootton (1972), Sharov (1973), Kukalová-Peck & Peck (1976), Sinitshenkova (1979), and Kukalová-Peck (1978, 1983). However, some of the interpretations and conclusions formulated by these authors are inconsistent, partly contradictory or based on *a priori* assumptions of a presumed aquatic lifestyle and modes of development, rather than strictly empirical evidence. We have therefore focused our effort on the verification of facts by additional observations from selected fossils, reaching the following conclusions. The available data from immature palaeodictyopterans document either young nymphal or late subimaginal instars. In early instars, the small wings are directed backwards and were rather broadly connected and often coupled with the corresponding thoracic tergite. Therefore, these rudimentary wings, with already well-developed corrugated venation, probably allowed only limited movement or were even fixed. Later instars are discernible by the wings becoming narrower basally and simultaneously having clearly developed joints, which probably allowed for some degree of movement. When the wings are positioned nearly perpendicular to the body axis, the immature insect is considered to have been a subimago. Thus, the recognition of immature stages is based on the angle of the wings/wing pads to the longitudinal body axis, even though it should properly be based on the presence of functional genitalia, but these are unfortunately poorly or not preserved and have failed to aid any reconstructions. Another point is the significant thickness of the wing membrane in nymphal and subimaginal instars of palaeodictyopterans, often explained as the retention of hypodermal tissue essential for the wing moult and in contrast to adult wings (Sinitshenkova, 1979). The gradual growth of nymphal wing pads during ontogeny, with subsequent narrowing of the wings in subimaginal stages, was proposed by Sharov (1973), Kukalová-Peck (1978, 1983), and others. However, evidence from the development of other structures such as the ovipositor contradicts the conclusion of gradual growth in at least some groups of Palaeodictyoptera. For example, a re-examination of *Paimbia fenestrata* Sinitshenkova (Tchirkovaeidae, paratype no. 2293/6) reveals a nymph with a considerably large body, reaching a length similar to the adult (about 45 mm), but bearing wing pads that are still directed backwards, which would suggest it was an early instar. However, it simultaneously has a well-developed, endophytic ovipositor that would support the notion of it being a later nymphal instar, if not a subimago. Prokop *et al.* (2013) described several specimens tentatively assigned to Breyeriidae that possessed small nymphal wing pads nearly perpendicular to the thorax, and which can hardly be considered as subimaginal. Thus, these examples demonstrate an inconsistency for the gradual model of wing development for at least some members of Palaeodictyoptera. On the other hand, in extant Ephemeroptera and Odonata, wings develop in sheaths until the last instar moults to the subimago in Ephemeroptera or to the adult in Odonata. Throughout the various nymphal instars, the wing pads or wing sheaths remain comparatively small, while the wings inside these sheaths grow to their final size, especially during the last few instars. The tissue of the wings is excessively folded inside the wing sheaths, due to the strongly restricted available space. This mode of growth, which is also found in all nonholometabolous Neoptera (Heming, 2003), possibly had the advantage that the nymphs are not hindered in their movements by the growing and probably functionless wings. Furthermore, the growing wings are well protected from any physical damage and permit the immatures to enter into small crevices for protection without jeopardizing their developing wings. External characteristics of such wing sheaths are the absence of a clearly visible venation, the more or less fixed position, and the fact that they do not grow in proportion to the rest of the animal, especially if compared with the final, adult size of the wings. In *Bizarrea*, the characters indicating its position among Spilapteridae, together with its size and the comparatively small size of the thoracic wing pads, imply that in this species the mode of growth of the wings was similar to what is known from modern palaeopterous insects and has the advantage of not requiring unique, *ad hoc* developmental modes for Paleozoic taxa.

Conclusions

The fossils preserved in sphero-siderite nodules from Mazon Creek represent an extraordinary source of knowledge on diversity and morphological disparity of Late Carboniferous insects. *Bizarrea obscura* **gen.n.** et **sp.n.** shows clear diagnostic traits for placement within Palaeodictyoptera, such as the rostrate mouthparts in the form of long stylets, features rarely preserved in their immature stages. Description of an additional specimen of the adult of *Homaloneura* cf. *dabasinskasi* Carpenter provides supplementary data regarding the morphology of thoracic and abdominal structures. Furthermore, we consider *B. obscura* as representative of Spilapteridae and we note the following shared features with *H.* cf. *dabasinskasi*: enlarged prothoracic lobes (winglets), heteronomous meso- and metathoracic wing pads or wings with corrugated venation, a slender abdomen with segments having a unique subdivision with two transverse sulci and pointed laterotergites. An alternative hypothesis for the placement of *Bizarrea* among Homoiopteridae is also considered on the basis of its large body size and relatively short wing pads, but these alone are considered weak evidence for such an assignment. Our re-examination of various palaeodictyopteran immatures shows weak support for the notion of gradual development of wings during ontogeny, in stark contrast to the conclusions of previous studies, and the growth of wing pads during postembryonic development for these groups is newly reconsidered.

Our comparative study of the morphology of abdominal segments using both light and environmental scanning electron microscopy (ESEM) reveals the presence of subcircular or oval, sclerotized structures beneath the nymphal laterotergites I–VII, ?VIII. The interpretation of these structures as abdominal tracheal gills with the laterotergites functioning as gill covers, and thereby supporting an aquatic lifestyle such as that known in extant mayflies (Ephemeroptera) is not supported. These structures are well delimited and lack any emerging filaments or even traces of such, and instead the morphology is that of abdominal spiracles, indicative of a terrestrial or semi-aquatic habitat for these immatures. Comparison with modern insects with similar life histories demonstrates a corresponding position and morphology of spiracles in moss bugs (Coleorrhyncha: Peloridiidae) living in habitats with high humidity, or in some onisciform beetle larvae such as Silphidae and Lampyridae (Coleoptera). Moreover, our re-examination of the enigmatic Vogesonymphidae (Protereismatina), known from the Middle Triassic of Grès à Voltzia in France, confirms the presence of homologous structures, also interpreted as spiracles. Thus, our discovery supports the parallel coexistence of ancestrally terrestrial or semi-terrestrial lineages of Ephemerida in early Mesozoic ecosystems. Despite these discoveries, considerably more data are needed from a broader diversity of Paleozoic insects before final conclusions can be reached regarding the complete life history of such extinct lineages.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12148

Figure S1. *Homaloneura* cf. *dabasinskasi* Carpenter, 1964: (A, B) frontal views of plate and counter-plate of PE 11268 reconstructed from micro-CT data, scale = 10 mm; (C) virtual three-dimensional model of counter-plate (c.f. B), resolution reduced by factor 4 to reduce file size; click the image to activate the three-dimensional view.

Figure S2. *Bizarrea obscura* **gen.n** et **sp.n.**: (A–C) frontal, lateral and backward views of holotype PE 11269 reconstructed from μCT data, scale = 10 mm; (D–F) sections through the specimen in three different perpendicular cutting-planes – note the homogeneous appearance of the interior of the concretion without any indication of fossil remains; (G) virtual three-dimensional model of specimen (cropped at the sides to reduce file size) – click the image to activate the three-dimensional view.

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