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To be or not to be: postcubital vein in insects revealed by microtomography

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Short title: PCu vein in insects

Abstract.

To better understand insect evolution, fossils – mainly known by their wings – must be used as terminals in phylogenetic analyses. Such analyses are, however, rarely performed because of a lack of consensus on the homology of venation in insects. Authors do not agree with current concept on the exact number and the identity of the main veins. Here, we confirm the presence, questioned since the early twentieth century, of an independent main postcubital vein (PCu) between the cubital and anal veins (29 fossil and extant examined orders, >85% of observed insects). The vein PCu corresponds to the so-called vein 1A or first anal vein. It is easily

identified by the unique shape of its bulla. It may have several branches, and be partially fused with the cubital and anal veins. Once the PCu vein identified, we reconsidered as an example the particular case of the Phasmatodea, showing that extant stick insects have a unique venation among insects, with a reduced median vein, and a simple cubital vein, adjacent or fused to the PCu. This study is a new approach towards resolving wing vein homology issues, crucial for future large-scale phylogenetic analyses in insects combining extant and extinct taxa.

Introduction

Although thousands of fossil insects have been described over the last twenty years, these extinct taxa are included in phylogenetic analyses only rarely enough (Misof *et al.*, 2014; Wang *et al.*, 2016). They are merely used for their temporal dimension as calibration points in dating estimates, whereas they can also bring original evolutionary evidence (i.e. new states of characters, new combinations of character states, new taxa). Taking this evolutionary information into account, and thus taking full advantage of those fossils, requires advances in primary homology of wing veins. Indeed, as the best-preserved structures of fossil insects, wing veins are critical to better understand the deep-past evolution of insects – as recently shown for some orders (e.g., Nel *et al.*, 2012; Prokop *et al.*, 2014, 2018; Jacquelin *et al.*, 2018). Yet, despite two centuries of efforts, the homologies of wing veins remain an open question. Authors do not agree on the exact number of main veins (see below), let alone their homologies (e.g., Comstock & Needham, 1898, 1899; Snodgrass 1935; Kukalová-Peck, 1991).

Wings are complex structures containing nerves, sensillae, and tracheae, all more or less connected to veins (Chapman, 1998). Veins can fork, vanish, be fused (Jacquelin *et al.*, 2018), or change their convexity (a convex vein, when seen from above, is in a higher position than a concave one), so many modifications that may explain why no consensus yet exists as to the number and identity of insect veins. From six to eight main longitudinal veins are thus

classically considered in insect wings, the area between the cubital and anal complexes being the main area of conflict (Table 1).

Among those conflicts (Table 1), the vein between the cubital and anal complexes has been identified either as a vein named postcubital or empusal vein (PCu or E), independent of the cubital or anal stems (e.g., Lameere, 1922; Snodgrass, 1935; Hamilton, 1972; Emeljanov, 1977), or as the first anal vein attached to the anal stem (e.g., Comstock & Needham, 1898, 1899; Wootton, 1979; Kukalová-Peck, 1992). Authors brought various evidences using different criteria to refine their homology hypotheses (e.g., tracheae, axillary sclerites, folds of wings). They also recognized that all of these criteria, when used individually, have defects (Hamilton, 1972) so that no hypothesis has received unanimous support (e.g., Matsuda, 1970 *contra* Wootton, 1979). Thus, the exact nature and the existence of an independent postcubital vein between the cubital and anal veins remain unsolved and hinder sound homology hypotheses at the largest insect scale.

Consequently, several venation groundplans exist and are currently used to describe insect venation. Most authors use the venation terminology proposed by Comstock & Comstock (1895), modified by Comstock & Needham (1898, 1899), and summarized by Comstock (1918). Kukalová-Peck (1991) proposed a second groundplan also currently used. A third proposal of groundplan with a further PCu vein between Cu and A, originally proposed by Lameere (1922) (PCu as ‘Pénultième’ vein) and partly followed by Forbes (1943) (PCu as Plical vein or Pl), was supported by Snodgrass (1935), Matsuda (1970), Emeljanov (1977), and Brodsky (1994). It was followed incidentally for Trichoptera and Lepidoptera (Ivanov, 1985, 1995), more frequently for Hemiptera (e.g., Franielczyk-Pietyra & Wegierek, 2019), and very recently for Blattodea (Li *et al.*, 2018). The PCu vein corresponds to the anterior anal vein (AA) of Kukalová-Peck (1991) and to the first anal branch of Comstock (1918), as already noticed by Séguy (1959: 50) (Table 1).

Table 1 : Comparison of different wing veins nomenclatures, with influence on the primary homologies. Terminology: a specific color was assigned for each homologous structure, viz., precostal stripe (PC), costa (C), subcosta (Sc), radius (R), sector (S), media (M), cubital (Cu), plical (P), postcubitus (PCu), empusal (E), anal (A), jugal (J).

Comstock & Needham (1898)	--	C	Sc	R		M	Cu		1st A	A	--
Snodgrass (1935) Emeljanov (1977)	--	C	Sc	R		M	Cu		PCu	A	--
Hamilton (1972)	--	C	Sc	R	S	M	Cu	P	E	A	--
Kukalová-Peck (1992)	PC	C	Sc	R		M	Cu		AA	AP	J

The origin of veins at the extreme base of wings must be observed to clarify vein homologies and resolve incongruities between the different venation patterns. Those observations are now possible because of adequate tools such as microtomography methods. Here, we use these tools in addition to direct optical observations to investigate the presence of the postcubital vein in the neopteran clades and in the Palaeodictyoptera fossils. We clarify the homologies of wing veins for those insects and illustrate the aftermath of this clarification using Phasmatodea, whose unusual venation has always been challenging (Ragge, 1955; Nel & Delfosse, 2011; Wang *et al.*, 2014).

Material and methods

Wing venation terminology and color code:

precostal stripe (PC), costa (C, in cyan), subcosta (Sc, in red), radius (R, in magenta), media (M, in blue), cubital (Cu, in yellow), postcubitus (PCu, in white), anal (A, in black), jugal (J). Each vein (x) is supposed to have a (convex) anterior branch (xA) and a (concave) posterior branch (xP) (Lameere, 1922; Kukalová-Peck, 1991). The bulla of the vein (x) is identified as xB.

Criteria for venation homology:

many criteria have been used to identify and postulate homology hypotheses on insect wing veins: the relative positions of veins, their polarities (convexity vs. concavity), the potential

correspondence between the courses of tracheae (air transport) and veins (hemolymph transport), or the structures of the extreme wing bases (see discussion on the interests and limits of these criteria in Desutter-Grandcolas *et al.*, 2017). Snodgrass (1935), Emeljanov (1977), Brodsky (1994), and Li *et al.* (2018), who all recognized a postcubital vein (PCu), based their results on alleged correspondences between the courses of the tracheae ‘Cu’, ‘PCu’ and ‘A’ in the nymphal wing pads and in adult wings, being basally well separated in many insects. Because counter-examples of such correspondences have been demonstrated (Fraser, 1938; Smart, 1956; Whitten, 1962; Wootton, 1992), this criterion is insufficient to accurately establish the existence of the PCu vein.

Most often, only direct observations with optic binoculars have been used but they are limited by the intricate and hidden structures located at the base of the wings. Recently, the advents of X-ray microtomography methods (e.g., μ CT-scan, Synchrotron) blew a technological lock to answer problems of insect venation (Desutter-Grandcolas *et al.*, 2017; Jacquelin *et al.*, 2018). After these studies, a main vein is considered as independent from the other main veins if its own base, i.e. its ‘bulla basivenale’, is independent of all other bullae, even though all the bullae share a common base, corresponding to the main cavity of the insect thorax. These bullae are hidden inside the very base of the wing and they generally differ from the external plates or sclerites of the wing base. With X-ray microtomography methods and 3D reconstruction based on non-automatic careful segmentation, bullae can be distinguished and each of them related to a main vein.

Taxonomic sampling:

We observed 74 extant and fossil specimens from all the Pterygota superorders (Palaeodictyoptera, Ephemeroptera, Odonatoptera, Polyneoptera, Acercaria, Holometabola). Nine of them were scanned with X-ray microtomography. A complete list of the observed specimens and technical characteristics of scans are given in supplementary material (see Supplementary tables 1,2).

We tested the variability of the structures of the wing base in three groups, viz. Orthoptera, Dictyoptera, and Phasmatodea. They were selected because, before conducting this study, we had already realized several observations in those three taxa. We found a high intraordinal stability for all veins including the PCu that appeared as a constant character that varied very little in position and shape. It allows us to claim that the character state ‘presence of a PCu’ is stable within an order, except when drastic reductions of venation occur, as in Tridactylidae, a family of Orthoptera where only one or two veins are still present in the wings. Consequently, the observation of few specimens is sufficient to evaluate the presence / absence of a PCu. Observed extant specimens were chosen following three main criteria: we selected specimens that were numerous in the MNHN collections or easily available because we often needed to damage the insects before observing them efficiently (e.g., we removed the scales of Lepidoptera or cut the pronotum of cockroaches and Orthoptera); we preferred large specimens to make observations easier; we used a phylogenetic criterion when observing multiple insects from the same order to maximize the diversity (e.g., specimens from different suborders of Plecoptera, Coleoptera, Trichoptera). Also, we used as many fossils as possible although their phylogenetic placements are often unclear and their wing bases are frequently not preserved. Fossils are indeed mandatory for extinct clades such as Palaeodictyopterida and Permopsocida. They are also useful to document past venation patterns.

Ancestral state reconstruction:

We used the parsimony method with Winclada v.1.00.08 (Nixon, 2002) to reconstruct the ancestral states for the character ‘presence of a PCu’ in insects. We used a simplified tree derived from the phylogeny of Wipfler *et al.* (2019: Fig. 1), with ‘Protelytroptera’ as sister group of Dermaptera (Béthoux *et al.*, 2016), Palaeodictyopterida within Palaeoptera, Hypoperlida as sister group of crown Acercaria (Prokop *et al.*, 2017) and Permopsocida as sister group of (Hemiptera + Thysanoptera) (Huang *et al.*, 2016). We performed two ancestral state

147 reconstructions: one with the state ‘?’ for taxa we are unsure about the presence of a PCu (i.e.
 148 taxa with reduced wings or venations – see below); one with the state ‘0’ (i.e. absence) for those
 149 taxa. The former corresponds to our current state of knowledge; it is thus our main result. The
 150 latter was performed only to assess whether our conclusions about PCu origin would still be
 151 supported if those problematic taxa truly lack a PCu (this coding is however highly hypothetical
 152 at this stage).

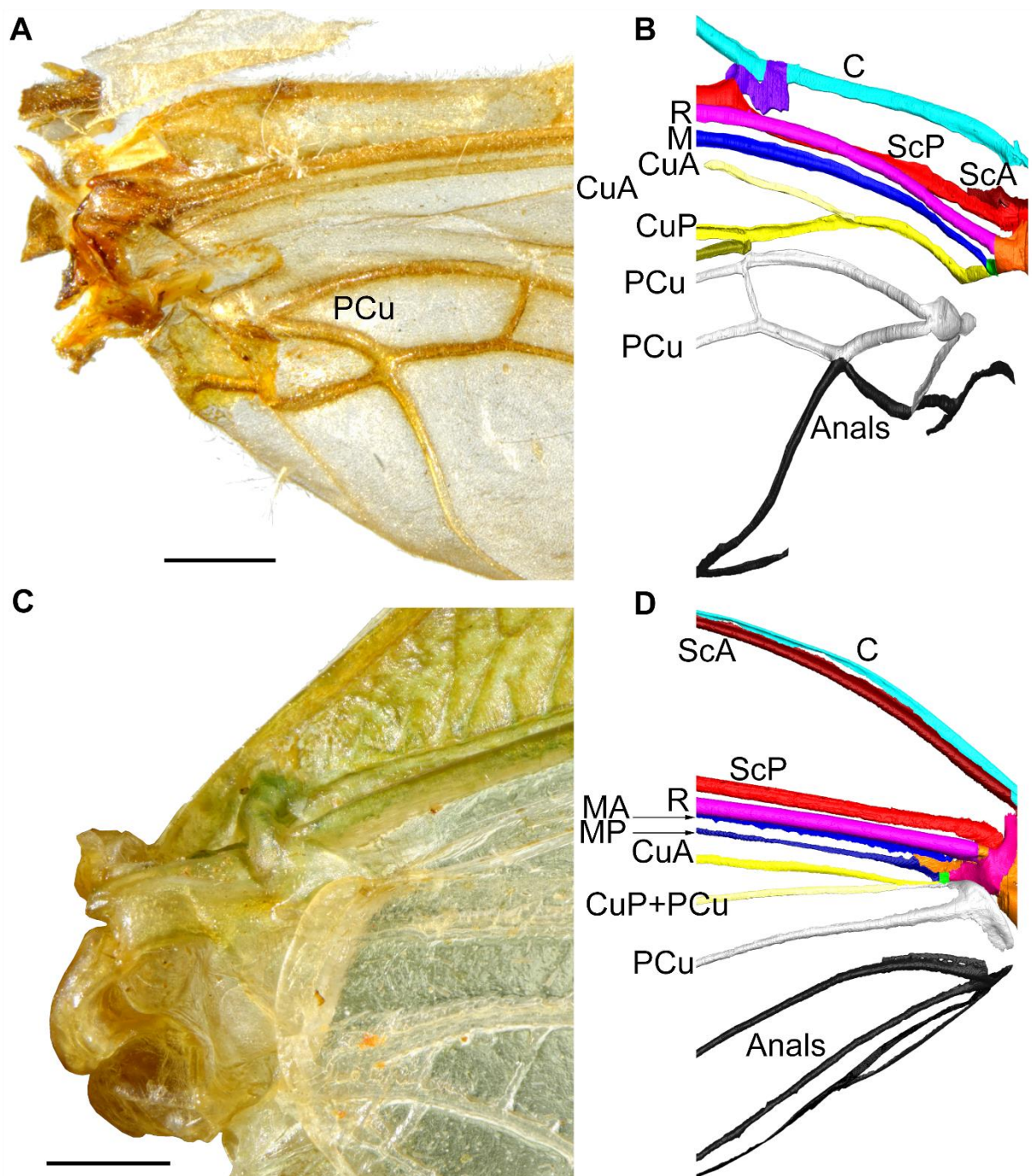


Fig. 1 : Forewing bases, photographs and 3D reconstructions, dorsal views. (A-B) *Plecoptera Perla marginata* (Panzer, 1799); (C-D) *Mantodea Stagmatoptera supplicaria* (Stoll, 1813). (copyright Thomas Schubnel). Scale bars = 1 mm.

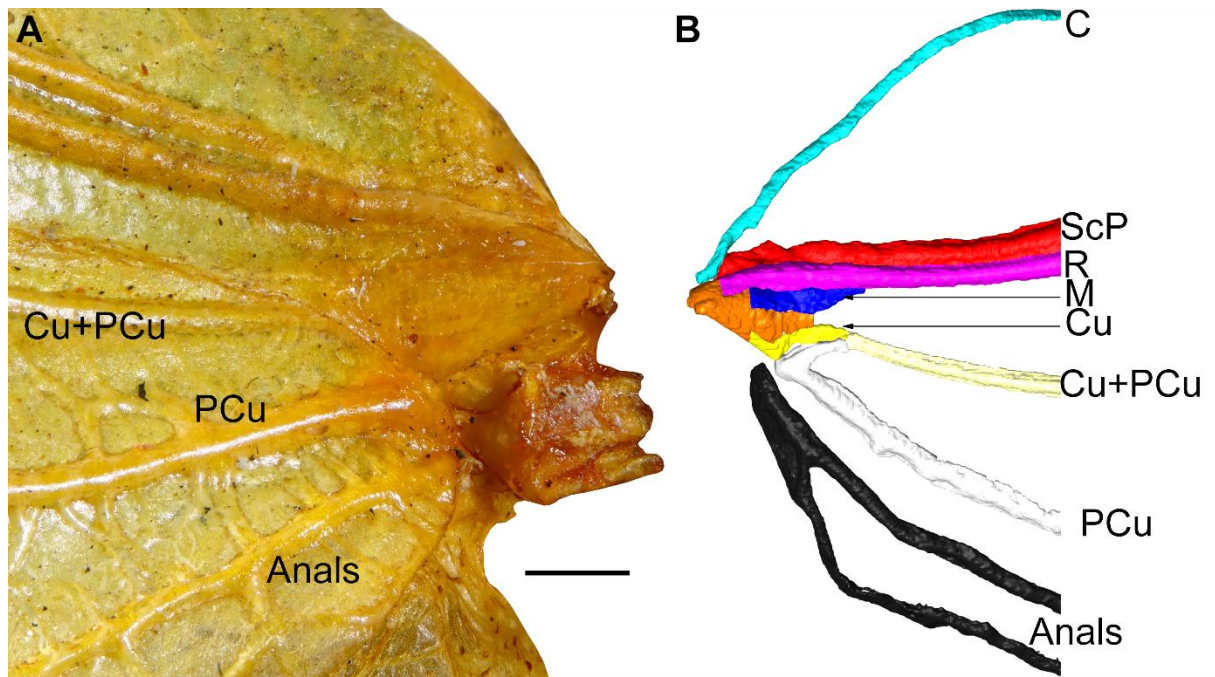


Fig. 2 : Forewing base, dorsal views. *Phasmatodea Phasma gigas* (Linnaeus, 1758). A, photograph; B, 3D reconstructions. (copyright Thomas Schubnel). Scale bars = 1 mm.

Results and Discussion

Bullae vs external sclerites:

The argument of Wootton (1979: 90) for a rejection of the hypothesis of an independent PCu does not stand because it is based on the supposed origin of the veins on the external sclerites. Yet, it is generally not the case after our X-ray microtomography observations: veins emerge from bullae, not from external sclerites. Odonatoptera, Ephemeroptera, and Palaeodictyoptera are exceptions with veins emerging from bullae and also connected to the basal sclerites (Prokop et al., 2018; Jacquelin *et al.*, 2018).

Identification of the PCu from its characteristic bulla:

With the microtomography tools and 3D reconstruction by segmentation, we observed an independent bulla between the bullae of the cubital and anal veins in representatives of the following orders: stoneflies (Plecoptera) (Figs 1A-B, Supplementary Fig. 1), mantises (Mantodea) (Figs 1C-D, Supplementary Fig. 2), cockroaches (Blattodea), katydids, crickets, grasshoppers (Orthoptera), phasmids (Phasmatodea) (Figs 2A-B), webspinners (Embiopoda), and beetles (Coleoptera). This bulla is characterized by its large, domed, convex, often

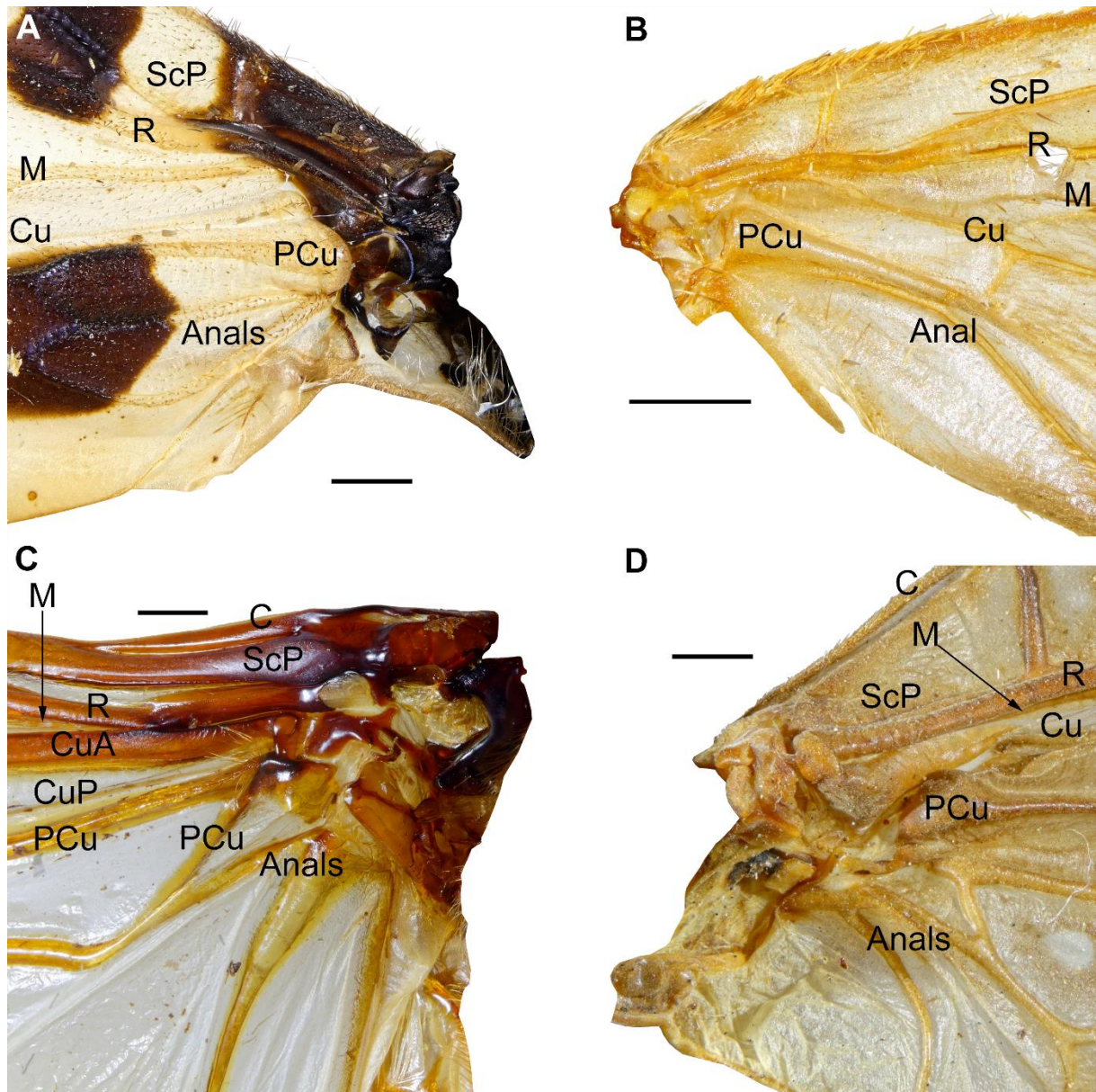


Fig. 3 : Forewing bases, photographs, dorsal views. A, *Trichoptera Semblis phalaenoides* (Linnaeus, 1758); B, *Lepidoptera Korscheltellus lupulinus* (Linnaeus, 1758); C, *Coleoptera Oryctes* sp.; D, *Megalopectera Corydalis* sp.; (copyright Thomas Schubnel). Scale bars = 1mm.

posteriorly curved structure, a set of features that allowed us to properly recognize it with a binocular microscope (Figs 1A,C, 2A, 3-6).

Presence of the PCu in most winged insects:

Because of its peculiar set of features and of its relative position, the PCu can be easily identified with a binocular microscope. In this way, we identified a bulla with the same shape directly posterior to cubital veins in true bugs (Hemiptera: Sternorrhyncha Psyllidae, Auchenorrhyncha Cicadellidae, Cercopidae, Fulgoridae, Cicadidae; Heteroptera Pentatomidae) (Figs 5A-B), in which it corresponds to the bulla of the vein currently named PCu after

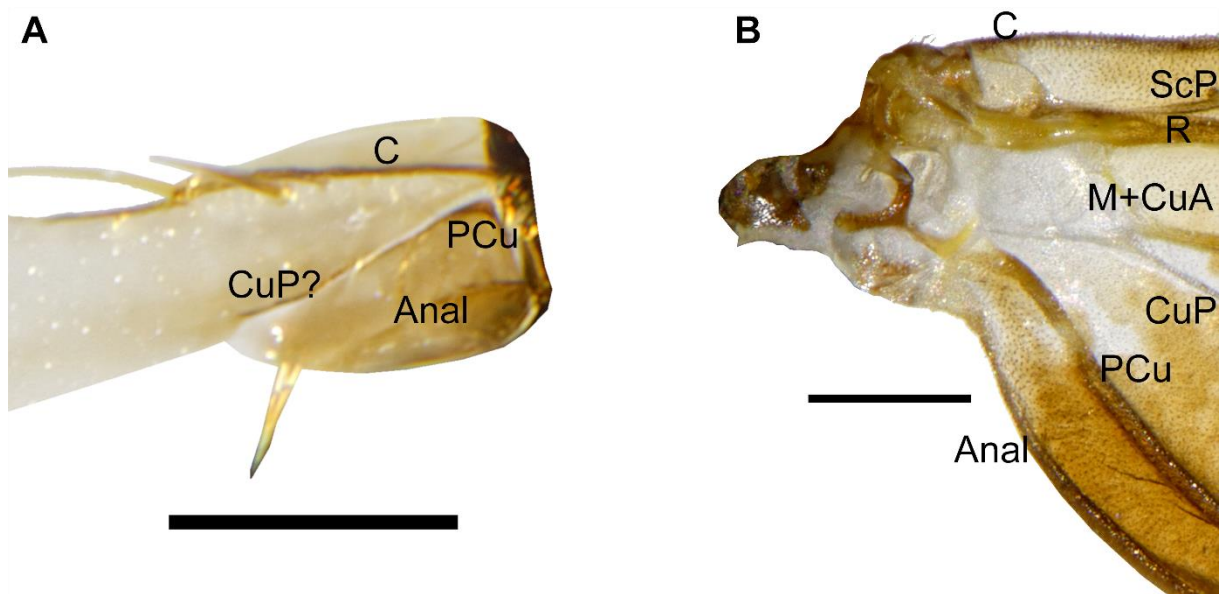


Fig. 5 : Forewing bases, photographs, dorsal views. A, Psocodea: Liposcelididae *Loensia fasciata* (Fabricius, 1787); B, Thysanoptera: Phaeothripidae *Megalothrips delmasi* Bournier, 1956. (copyright Thomas Schubnel). Scale bars = 0.25 mm.

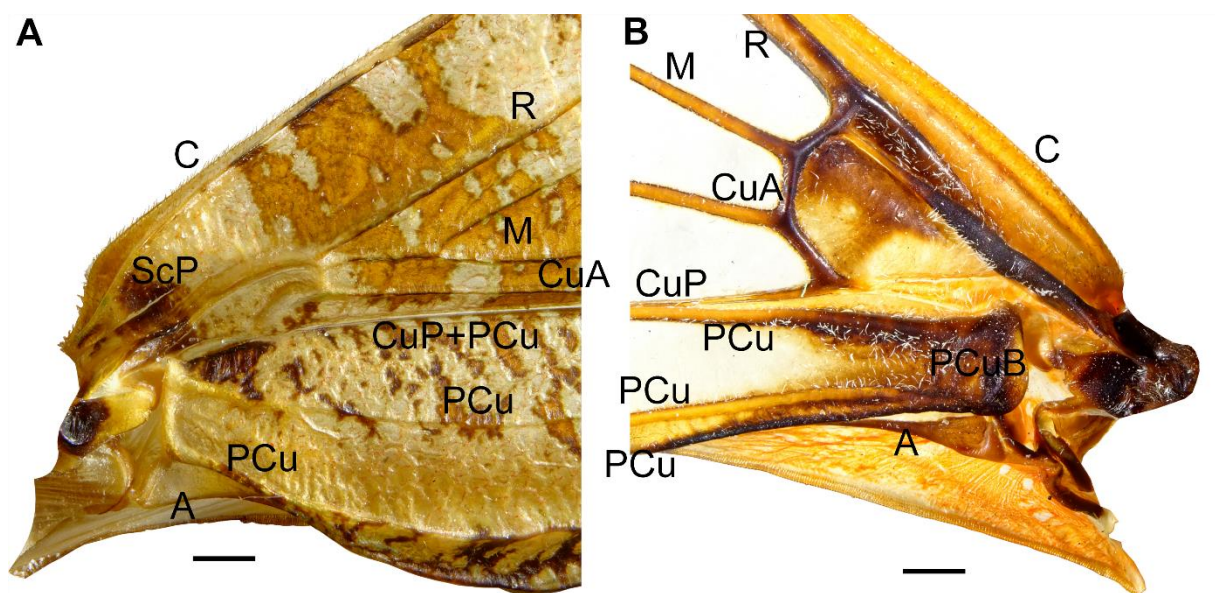


Fig. 4 : Forewing bases of Hemiptera, photographs, dorsal views. A, Fulgoridae *Phrictus auromaculatus* Distant, 1905; B, Cicadidae *Lyristes plebejus* (Scopoli, 1763). (copyright Thomas Schubnel). Scale bars = 1 mm.

176 Emeljanov (1977) with its characteristic domed structure. We also observed this bulla in
 177 termites (Isoptera: Mastotermitidae, other termites have reduced venations), scorpionflies
 178 (Mecoptera: Panorpididae, Bittacidae), caddisflies (Trichoptera: Phrygaenidae, Molannidae,
 179 Sericostomatidae, †Necrotaulidae, Philopotamidae) (Fig. 3A), moths (Lepidoptera: Hepialidae,
 180 Micropterigidae, and Agathiphagidae, confirmed from Ivanov (1995: figs 3-4) for the two later)
 181 (Fig. 3B), lacewings (Neuroptera), beetles (Coleoptera: Cerambycidae, Scarabaeidae) (Fig.

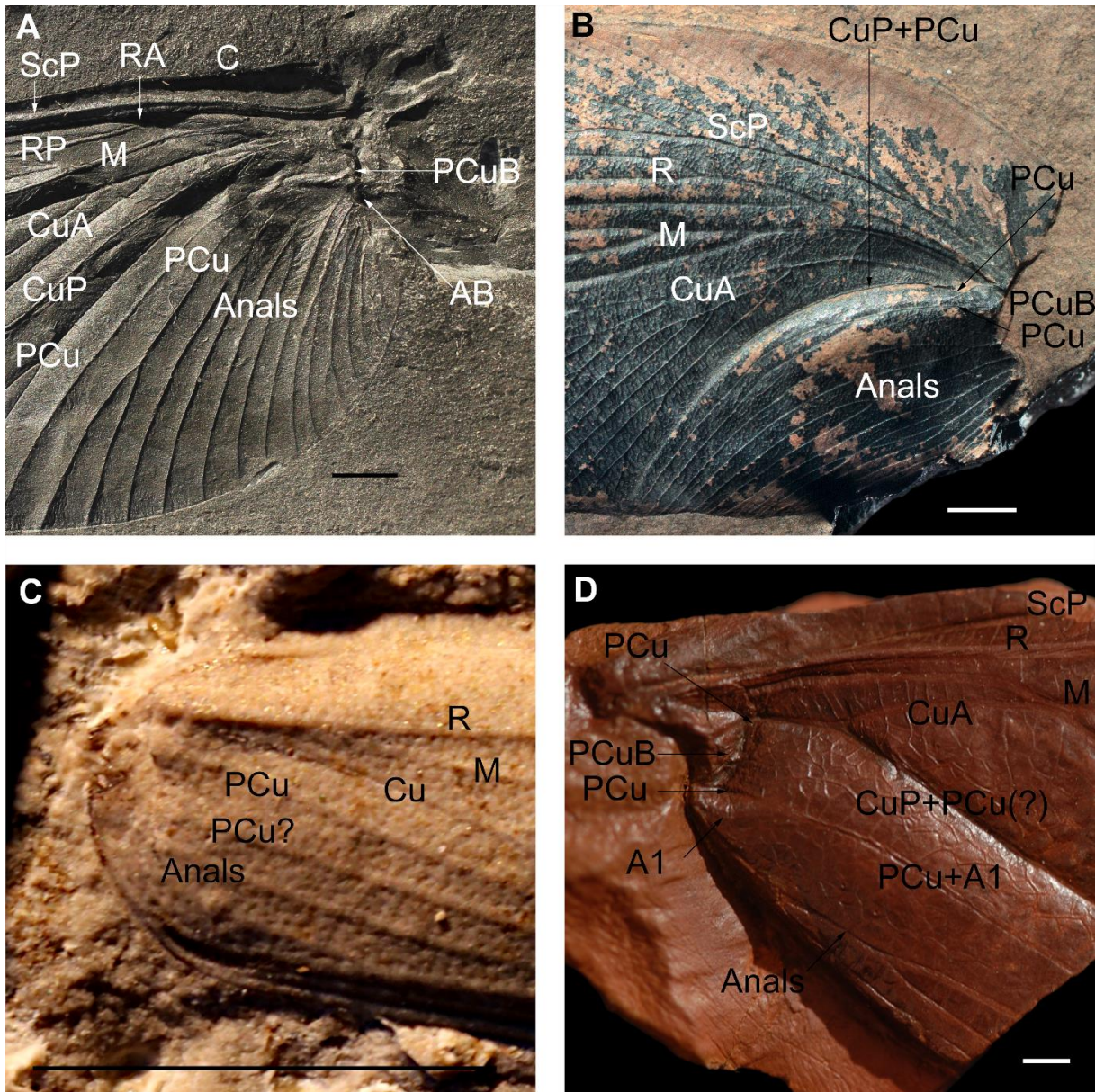


Fig. 6 : Wing bases of Paleozoic insects. A, †*Palaeodictyoptera* *Dunbaria quinquefasciata* (Martynov, 1940), hindwing, PIN No. 1631/311; B, *Dictyoptera* *Phyloblatta gaudryi* Agnus 1903, forewing, MNHN-F-R51244; C, †‘*Protelytroptera*’ *Protelytron furcatum* Carpenter, 1939, Paratype MCZ 3885; D, †*Paoliidae* *Darekia sanguinea* Prokop et al., 2012, forewing, MP ISEA I–F/MP/1488/14a/08. (copyright A, D Jakub Prokop; B, Gaëlle Doitieu E-recolnat, MNHN; C, Museum of Comparative Zoology, Cambridge, USA). Scale bars = 2 mm.

3C), dobsonflies (Megaloptera: Corydalidae, Sialidae) (Fig. 3D), snakeflies (Raphidioptera),

lice and thrips (Psocodea and Thysanoptera) (Fig. 4).

We also identified the PCu bulla in the following fossils: a “six-winged insects” (Palaeodictyoptera) (Fig. 6A), stem Dictyoptera (roachoids) (Fig 6B), Paoliidae (Fig. 6D), Archaeorthoptera, Hypoperlida and Permopsocida. Extant Dermaptera have a much reduced forewing venation that is hard to interpret but it is not the case of their putative stem group, the

‘Protelytroptera’ (Béthoux *et al.*, 2016). A representative of the ‘Protelytroptera’ shows a strongly convex and curved structure from which two PCu veins emerge (Fig. 6C). In all the observed insects, only two or three branches emerge from the PCu bulla, and these are generally all convex, see the new proposal of pattern of venation Fig. 7A.

The only insects for which this vein could not be formerly identified are angel insects (Zoraptera) and dragonflies (Odonata). Zoraptera have a very simplified venation with only three main veins, none of which emerge from a bulla typical of the PCu. The absence of the particular PCu structure could be however explained by the reduced ‘anal’/posterior area of the wing. In the Odonata there is also no particular convex and curved bulla posterior of the cubital veins; we observed however a vein forked into an anterior convex branch and a posterior concave branch that emerges from a bulla that is in a very basal position (Jacquelin *et al.*, 2018); posterior to this vein, there is only a membranule (small membrane at the postero-basal angle of wing) with very weak veinlets (in some fossil Isophlebioidea) or no vein at all. This forked vein corresponds to the anal vein followed by the ‘jugal’ area of the Polyneoptera. Thus the postcubital vein and bulla could be absent in the order Odonata.

Overall, we observed a PCu in 63 of the 74 observed Pterygota, covering 23 of the 29 examined orders, using both microtomography tools and stereomicroscopy. Only two orders lack this vein (Zoraptera and Odonata), and we have yet to clarify the situation for three extant orders (Ephemeroptera, Diptera, Hymenoptera).

Taxa with unclear results:

The observation of wing venations revealed challenging for Ephemeroptera, Hymenoptera, and Diptera. We were thus unable to unambiguously conclude about the presence of a PCu for those taxa. Surprisingly, CT scan analysis was particularly difficult for Ephemeroptera and it was not possible to conclude unambiguously using only stereomicroscopy observations. In Odonata, extant taxa have no visible PCu, but we currently lack Paleozoic Odonoptera showing the delicate structures of wing base to conclude whether PCu has been lost in crown-Odonoptera

or never acquired in this lineage. Extant and fossil Ephemeroptera and Odonatoptera will thus be investigated more in depth in a second step. In Palaeodictyopteroid orders, the situation is unsettled for three of the four orders (Megasecoptera, Dicliptera and Diaphanopteroidea, sensus Prokop & Engel, 2019) due to the lack of adequate fossils. In Hymenoptera, the PCu and anal veins are apparently strongly reduced, whereas in Diptera the anal veins, PCu and Cu seem to be fused at wing base. Our observations must be confirmed with additional 3D tomography reconstructions. These clarifications will not, however, change our conclusions on the origin of the PCu because of the derived positions of Hymenoptera and Diptera.

Ancestral state reconstruction – origin of the PCu:

Parsimoniously mapping the character ‘presence of the PCu’ on a phylogenetic tree, we observed that the ‘ancestral’ presence of the PCu in all Pterygota is the most parsimonious hypothesis (only two reversals) to explain insect venation (Fig 7B); the opposite hypothesis (‘ancestral’ absence of PCu in Neoptera) implies four independent gains of the PCu. Because the existence of a PCu is still unclear for a few taxa, we hypothesized that those taxa lack a PCu and run a second ancestral state reconstruction. In this very hypothetical scenario, an ancestral presence of the PCu for Pterygota cannot be ruled out (ambiguous reconstruction for the Pterygota and Neoptera nodes). This ambiguous reconstruction only holds if ‘Protelytroptera’ are monophyletic, which is unsure (Béthoux et al., 2016). If ‘Protelytroptera’ are paraphyletic, then the scenario with an ancestral presence of the PCu is more parsimonious. Overall, our results support a single origin of the PCu.

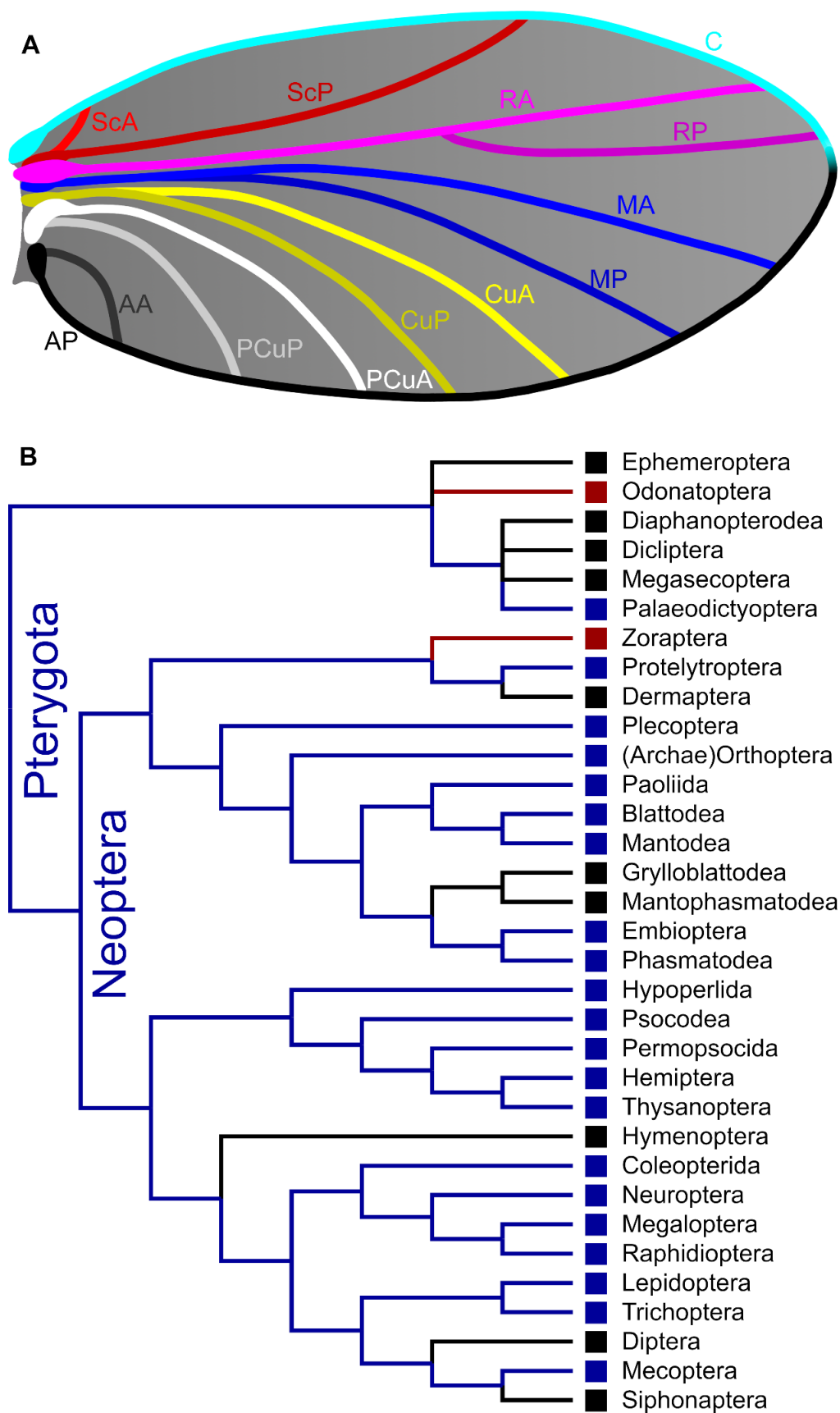


Fig. 7 : A, Proposed general pattern of insect wing venation with PCu veins. Posterior branches are in darker colors than anterior branches. B, Phylogenetic inference in parsimony of the presence of the PCu vein in Pterygota. Clades with PCu are in dark blue, those without are in red, and clades where the situation is unclear are in dark. (Archae)Orthoptera comprise extant Orthoptera and stem-lineage.

The PCu and its implications:

Our results support Snodgrass (1935), Emeljanov (1977), Brodsky (1994), and Li *et al.* (2018) about the existence of a postcubital vein, independent of the cubital and the anal veins. These three veins emerge from three independent bullae. We go further, however, demonstrating that a majority of orders have a PCu and that the situation is even more complex than supposed by these authors. They generally considered that the PCu is not fused or closely adjacent to the branch(es) of the cubital system. Emeljanov (1977) and Brodsky (1994) thought that the PCu is a simple vein, posterior to CuP, in all insects. Li *et al.* (2018) stated that the anal veins of Comstock–Needham should be interpreted as two independent main veins, their PCu and ‘vannal’ veins, on the basis of a supposed correspondence between the trachea and the veins in Blattodea. In fact, in many neopteran orders, the PCu is forked with its anterior branch appressed or partly fused to the CuP [e.g., Dictyoptera, for which Kukalová-Peck (1992) indicated a fusion between her ‘CuP’ and ‘AA1’ (= PCu)].

The presence of the PCu also implicates that the anal area is smaller than previously suggested, which has implication on the evolution of flight and notably on the ‘umbrella effect’ (*sensu* Wootton, 1992: 131): “the soft vannus is supported by a series of unbranched anal and jugal veins radiating from the base.” In Holometabola, the anal area is often reduced to only one vein. In Plecoptera, the anal area is strongly reduced in some species (e.g. *Filchneria olgae* (McLachlan, 1875)) and strongly developed with numerous veins associated to a reduced post-cubital area in others (e.g. *Eusthenia spectabilis* Westwood, 1832) (Fig. 8). In some groups, such as Blattodea and Palaeodictyoptera, in which the ‘anal area’ is considered as very developed, the existence of the PCu vein modifies the interpretation/identities/homologies of ‘anal area’ veins. The Palaeodictyoptera have numerous anal and post-cubital veins, while Blattodea have few post-cubital veins and several anal veins. So, the recognition of the PCu as an independent vein sheds a new light on some cases of evolutionary convergences for the ‘umbrella effect’ (Desutter-Grandcolas et al., 2005; Wootton, 1992).

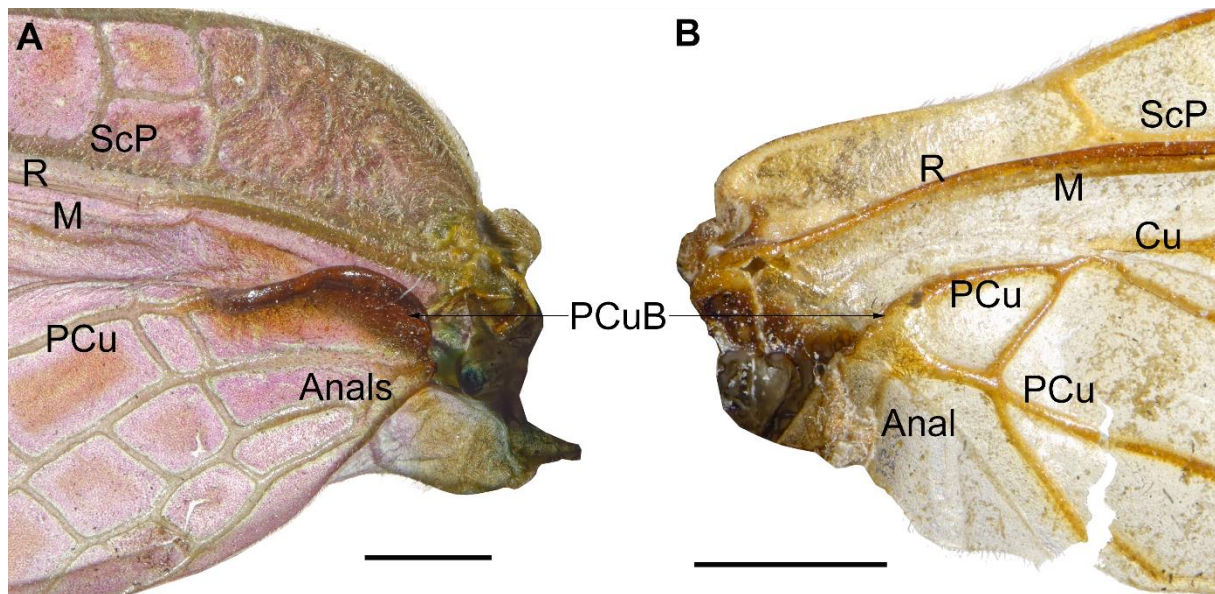


Fig. 8 : Forewing bases, photographs, dorsal views. Plecoptera. A, Eustheniidae *Eusthenia spectabilis* Westwood, 1832, Perlodidae *Filchneria olgae* (McLachlan, 1875). (copyright Thomas Schubnel). Scale bars = 1 mm.

The specific case of Phasmatodea:

The extant Phasmatodea show an unusual forewing venation in which the main veins are difficult to recognize. Ragge (1955) proposed a pattern based on a putative correspondence between veins and trachea, that was questioned by Nel & Delfosse (2011) and Wang et al. (2014) on the basis of the study of the venation of Mesozoic taxa currently attributed to the stem group of Phasmatodea. These last authors considered that these fossils had venations of archaeorthopteran type (sensu Béthoux & Nel, 2002), but this last pattern was not recovered in extant stick insects. After our CT reconstructions, extant stick insects have a median vein extremely reduced and appressed to the radial vein and a simple vein Cu, completely separated from the median and radial veins, either only present at the extreme wing base and immediately ending into the anterior branch of PCu (Fig. 2) (e.g., *Phasma gigas* (Linnaeus, 1758)), or independent from PCu (e.g., *Tropidoderus childrenii* Gray, 1833). The PCu structure consists in two clearly convex veins, emerging from a bulla in domed structure, independent of cubital and anal bullae. Previous authors considered these two PCu veins as cubital veins (Ragge, 1955), or anal veins (Nel & Delfosse, 2011). Thus the extant stick insects have an extremely particular forewing venation unique among all the Pterygota, with the median and cubital veins

nearly absent. This venation is completely different from the pattern of the Archaeorthoptera (incl. extant Orthoptera) that have a basal fusion of R, M, CuA, and a three-branched CuP. These results will be used to re-investigate the changes in the wing venation between the alleged Mesozoic stem Phasmatodea and the extant stick insects.

Conclusion

Three-D X-ray tomography and reconstructions are very efficient to help determining the homologies of wing venation among insects, as already shown in recent studies (Desutter-Grandcolas *et al.*, 2017; Jacquelin *et al.*, 2018). Here, it allowed us to demonstrate the existence of a PCu vein in most insect orders, and suggest that this structure most likely originated only once in insects. This result has important consequences for evolutionary analyses in these organisms. Until now, the same homologous vein was named AA1 for Orthoptera and PCu for Hemiptera for example, a situation hindering comparative analyses. The case of the stick insects shows that it should help to solve long-lasting evolutionary problems. It remains to determine if all Palaeoptera (extant and/or fossil) have a PCu vein or not, viz. the other Palaeodictyopterida orders Megasecoptera, Dicliptera, and Diaphanopterodea, the Carboniferous stem Odonatoptera and the fossil and extant Panephemeroptera. The presence of a PCu could constitute a synapomorphy of the Neoptera + Palaeodictyopterida, supported by the recently performed phylogenetic analysis of Sroka *et al.* (2015), and potentially absent in the Odonatoptera and Panephemeroptera. This result would be pivotal to continue unlocking crucial homology issues in insect wing venation, ultimately allowing integrating extant and fossil insects at an order level in phylogenetic analyses, a work impossible to achieve under the current state of the art.

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References

- Béthoux, O., Llamasi, A. & Toussaint, S. (2016) Reinvestigation of *Protelytron permianum* (Insecta; Early Permian; USA) as an example for applying reflectance transformation imaging to insect imprint fossils. *Fossil Records*, **20**, 1–7.
- Béthoux, O. & Nel, A. (2002) Venation pattern and revision of Orthoptera sensu nov. and sister groups. Phylogeny of Palaeozoic and Mesozoic Orthoptera sensu nov. *Zootaxa*, **96**, 1–88.
- Brodsky, A.K. (1994) The evolution of insect flight. Oxford University Press: Oxford / New York / Tokyo, xiii + 229 pp.
- Chapman, R.F. (1998) The insects structure and function. Chapter The head. Cambridge University Press, 4th Edition.

- 326 Comstock, J.H. 1918. The wings of insects. Comstock Publishing Co. (publ.) Ithaca, New York:
327 xviii + 430 pp.
- 328 Comstock, J.H. & Comstock, A.B. (1895) A manual for the study of insects. Ithaca, New York,
329 7–701.
- 330 Comstock, J.H. & Needham, J.G. (1898) The wings of insects. An introduction to the study of
331 the homologies of the wing-veins. *The American Naturalist*, **32**, 43–48, 81–89, 231–
332 257, 335–340, 413–424, 561–565, 769–777, 903–911.
- 333 Comstock, J.H. & Needham, J.G. (1899) The wings of insects. Chapter 4. The specialization of
334 wings by addition. *The American Naturalist*, **33**, 117–126, 573–582, 845–860.
- 335 Desutter-Grandcolas, L., Legendre, F., Grandcolas, P., Robillard, T., & Murienne, J. (2005)
336 Convergence and parallelism: is a new life ahead of old concepts? *Cladistics*, **21**, 51-
337 61.
- 338 Desutter-Grandcolas, L., Jacquelin, L., Hugel, S., Boistel, R., Garrouste, R., Henrotay, M.,
339 Warren, B.H., Chintauan-Marquier, I.C., Nel, P., Grandcolas, P. & Nel, A. (2017) 3-D
340 imaging reveals four extraordinary cases of convergent evolution of acoustic
341 communication in crickets and allies (Insecta). *Scientific Reports*, **7** (1) (7099), 1–8.
- 342 Emeljanov, A.F. (1977) Homology of wing structures in the cicada and the primitive
343 Polyneoptera. *Trudy Vsesofuznogo Entomologicheskogo Obshchestva* [*Proceedings of*
344 *Allunion Entomological Society*], **58**, 3–48. (in Russian)
- 345 Forbes, W.T.M. (1943) The origin of wings and venational types in insects. *American Midland*
346 *Naturalist*, **29**, 381–405.
- 347 Franielczyk-Pietyra, B., & Wegierek, P. (2019) The forewing of *Cacopsylla mali*
348 (Schmidberger 1836) (Hemiptera, Sternorrhyncha) - a morphological and histological
349 study. *Zoologischer Anzeiger*, **278**, 95–100.

350 Fraser, F.C. (1938) A note on the fallaciousness of the theory of pretracheation of the venation
 351 of Odonata. *Proceedings of the Royal Entomological Society*, London, (A), **13**, 60–70.

352 Hamilton, K.G.A. (1972) The insect wing, Part 2. Vein homology and the archetypal insect
 353 wing. *Journal of the Kansas Entomological Society*, **45**, 54–58.

354 Huang, Di-Ying, Bechly, G., Nel, P., Engel, M.S., Prokop, J., Azar, D., Cai, Chen-Yang, van
 355 de Kamp, T., Staniczek, A.H., Garrouste, R., Krogmann, L., dos Santos Rolo, T.,
 356 Baumbach, T., Ohlhoff1, R., Shmakov, A.S., Bourgoin, T., & Nel, A. (2016) New fossil
 357 insect order Permopsocida elucidates major radiation and evolution of suction feeding
 358 in hemimetabolous insects (Hexapoda: Acercaria). *Scientific Reports*, **6** (23004), 1–9.

359 Ivanov, V.D. (1985) Structure and function of wing articulation of caddisflies. 1. Ground plan.
 360 *Vestnik Leningradskogo Universiteta Biologiya*, **1985**, 3–12. (in Russian)

361 Ivanov, V.D. (1995) Comparative analysis of wing articulation in archaic Lepidoptera.
 362 *Entomological Review*, **74**, 32–53.

363 Jacquelin, L., Desutter-Grandcolas, L., Chintauan-Marquier, I., Boistel, R., Zheng, Daran,
 364 Prokop, J. & Nel, A. (2018) New insights on basivenal sclerites using 3D tools and
 365 homology of wing veins in Odonatoptera (Insecta). *Scientific Reports*, **8** (238), 1–7.

366 Kukalová-Peck, J. (1991) Fossil history and the evolution of hexapod structures. In *Insects of*
 367 *Australia*, I.D. Naumann, ed. (Melbourne: Melbourne University Press, CSIRO), pp.
 368 141–179.

369 Lameere, A. (1922) Sur la nervation alaire des insectes. *Bulletin de la Classe des Sciences*,
 370 *Académie Royale de Belgique*, (5), **8**, 138–149.

371 Li, X.-R., Zheng, Y.-H., Wang, C.-C. & Wang, Z.-Q. (2018) Old method not old-fashioned:
 372 parallelism between wing venation and wing-pad tracheation of cockroaches and a
 373 revision of terminology. *Zoomorphology*, **137**, 519–533.

374 Matsuda, R. (1970) Morphology and evolution of the insect thorax. *Memoirs of the*
375 *Entomological Society of Canada*, **102** (suppl. 76), 1–431.

376 Misof, B., Liu, S.-l., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware,
377 J., Flouri, T., Beutel, R.G., Niehuis, O., Petersen, M., Izquierdo-Carrasco, F., Wappler,
378 T., Rust, J., the 1KITE consortium (83 other authors), Wang, J., Kjer, K.M. & Zhou, X.
379 (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science*, **346**
380 (6210), 763–767.

381 Nel, A. & Delfosse, E. (2011) A new Chinese Mesozoic stick insect. *Acta Paleontologica*
382 *Polonica*, **56**, 429–432.

383 Nel, A., Prokop, J., Nel, P., Grandcolas, P., Huang, Di-ying, Roques, P., Guilbert, E., Dostál,
384 O. & Szwedo, J. (2012) Traits and evolution of wing venation pattern in paraneopteran
385 insects. *Journal of Morphology*, **273**, 480–506.

386 Nixon, K.C. (2002). WinClada, version 1.00. 08. *Published by the author, Ithaca, NY*, 734–
387 745.

388 Prokop, J., & Engel, M.S., 2019. Palaeodictyopterida. *Current Biology*, **29**, R307–R309.

389 Prokop, J., Pecharová, M., Nel A. & Hörschemeyer, T. (2018) The wing base of the
390 palaeodictyopteran genus *Dunbaria* Tillyard: Where are we now? *Arthropod Structure*
391 *& Development*, **47**, 339–351.

392 Prokop J., Krzeminski W., Krzeminska E., Hörschemeyer T., Ilger J.-M., Brauckmann C.,
393 Grandcolas P. & Nel A. (2014) Late Palaeozoic Paoliida is the sister group of
394 Dictyoptera (Insecta: Neoptera). *Journal of Systematic Palaeontology*, **12**, 601–622.

395 Prokop, J., Pecharová, M., Garrouste, R., Beattie, R., Chintauan-Marquier, I.C., & Nel, A.
396 (2017) Redefining the extinct orders Miomoptera and Hypoperlida as stem acercarian
397 insects. *BMC Evolutionary Biology*, **17** (205), 1–20.

- 398 Ragge, D.R. (1955) The wing-venation of the order Phasmida. *Transactions of the Royal*
399 *Entomological Society*, **106**, 375–392.
- 400 Séguy, E. (1959) Introduction à l'étude morphologique de l'aile des insectes. *Mémoires du*
401 *Muséum National d'Histoire Naturelle*, Paris, (A), *Zoologie*, **21**, 1–248.
- 402 Smart, J. (1956) A note on insect wing veins and their tracheae. *Quarterly Journal of*
403 *Microscopical Science*, **97**, 535–539.
- 404 Snodgrass, R.E. (1935) Principle of insect morphology. McGraw-Hill (publ.), New York, ix +
405 667 pp.
- 406 Sroka, P., Staniczek, A.H. & Bechly, G. (2015) Revision of the giant pterygote insect
407 *Bojophlebia prokopi* Kukalová-Peck, 1985 (Hydropalaeoptera: Bojophlebiidae) from
408 the Carboniferous of the Czech Republic, with the first cladistic analysis of fossil
409 palaeopterous insects. *Journal of Systematic Palaeontology*, **13**, 963–982.
- 410 Wang, Mao-min, Béthoux, O., Bradler, S., Jacques, F.M.B., Cui, Yingying & Ren, D. (2014)
411 Under cover at pre-angiosperm times: a cloaked phasmatodean insect from the Early
412 Cretaceous Jehol biota. *PlosOne*, **9** (3) (e91290), 1–10.
- 413 Wang, Yanhui, Engel, M.S., Rafael, J.A., Wu, Hao-yang, Rédei, D., Xie, Qiang, Wang, Gang,
414 Liu, Xiao-guang & Bu, Wen-jun (2016) Fossil record of stem groups employed in
415 evaluating the chronogram of insects (Arthropoda: Hexapoda). *Scientific Reports*, **6**
416 (38939), 1–12.
- 417 Whitten, J.M. (1962) Homology and development of insect wing tracheae. *Annals of the*
418 *Entomological Society of America*, **55**, 288–295.
- 419 Wipfler, B., Letsch, H., Frandsen, P.B., Kapli, P., Mayer, C., Bartel, D., Buckley, T.R., Donath,
420 A., Edgerly-Rooks, J.S., Fujita, M., Liu, Shanlin, Machida, R., Mashimo, Y., Misof, B.,
421 Niehuis, O., S. Peters, R.S., Petersen, M., Podsiadlowski, L., Schütte, K., Shimizu, S.,
422 Uchifune, T., Wilbrandt, J., Yan, E., Zhou, Xin & Simon, S. (2019) Evolutionary history

423 of Polyneoptera and its implications for our understanding of early winged insects.
424 Proceedings of the National Academy of Sciences, **116**, 3024–3029.
425 Wootton, R.J. (1979) Function, homology and terminology in insect wing. *Systematic*
426 *Entomology*, **4**, 81–93.
427 Wootton, R.J. (1992) Functional morphology of insect wings. *Annual Review of Entomology*,
428 **37**, 113–140.

Supplementary material

Material and methods

Abbreviations of institutions: MNHN (Muséum national d'Histoire naturelle, Paris); NIGP (Nanjing Institute of Geology and Paleontology, Academia Sinica, China); PIN (Laboratory of Arthropods, Palaeontological Institute, Russian Academy of Sciences, Moscow); MCZ (Museum of Comparative Zoology, Cambridge, USA); MP ISEA (Natural History Museum of the Institute of Systematics and Evolution of Animals, Polish Academy of Science, Kraków, Poland); IRSNB (Institut Royal d'Histoire Naturelle de Belgique, Bruxelles); UP (Université de Poitiers, Poitiers).

Observed specimens:

Supplementary table 1. List of observed specimens. All extant dried specimens are in the entomology collection of the Muséum national d'Histoire naturelle (MNHN).

Order	Species	X-ray scanned	PCu identified	Inventory number	Period
†Palaeodictyoptera	† <i>Dunbaria quinquefasciata</i> (Martynov, 1940)		Yes	PIN 1631/311	Permian
†Megasecoptera	† <i>Scytohymen extremus</i> Martynov, 1937		?	PIN 4987/113	Permian
Ephemeroptera	<i>Ephemera danica</i> Müller, 1764	UP	?	MNHN EP578	Modern
	<i>Oligoneuriella rhenana</i> (Imhoff, 1852)		?		Modern
	<i>Baetis</i> sp.		?		Modern
Odonata	<i>Aeshna cyanea</i> (Müller, 1764)		No		Modern
	<i>Aeshna isoceles</i> Müller, 1767	UP	No		Modern

†Protelytroptera	† <i>Protelytron furcarum</i> Carpenter, 1939		Yes	MCZ 3885	Permian
Zoraptera	<i>Zorotypus caudelli</i> Karny, 1927		No		Modern
Plecoptera	<i>Perla marginata</i> (Panzer, 1799)	ESRF	Yes	MNHN EP 4340	Modern
	<i>Eusthenia spectabilis</i> Westwood, 1832		Yes		Modern
	<i>Filchneria olgae</i> (McLachlan, 1875)		Yes		Modern
Panorthoptera	† <i>Cacurgus</i> sp.		Yes	MNHN F A 70497	Carboniferous
Orthoptera	<i>Brachytrupes</i> sp.		Yes		Modern
	<i>Chorthippus scalaris</i> (Fischer von Waldheim, 1846)		Yes		Modern
	<i>Gryllacropsis</i> sp.	MNHN	Yes	MNHN EO ENSIF 3080	Modern
	<i>Locusta migratoria</i> (Linnaeus, 1758)		Yes		Modern
	<i>Phymateus saxosus</i> Coquerel, 1861		Yes		Modern
	Trigonopteryginae sp.	MNHN	Yes	MNHN EO CAELIF 3000	Modern
Phasmatodea	<i>Phasma gigas</i> (Linnaeus, 1758)	MNHN	Yes	MNHN EO PHAS 1083	Modern
	<i>Phyllium</i> sp.		Yes		Modern

	<i>Tropidoderus childrenii</i> Gray, 1833		Yes		Modern
Embioptera	<i>Aposthonia ceylonica</i> Enderlein, 1912	ESRF	Yes	MNHN EP 4342	Modern
	<i>Berlandembia berlandi</i> (Navás, 1922)		Yes		Modern
†Paoliida	† <i>Darekia sanguinea</i> Prokop et al., 2012		Yes	MP ISEA I–F/MP/1488/14a/08	Carboniferous
Dictyoptera	† <i>Miroblattites costalis</i> (Laurentiaux-Vieira & Laurentiaux, 1987)		Yes	IRSNB No. 15.576	Carboniferous
	† <i>Phyloblatta gaudryi</i> Agnus 1903		Yes	MNHN-F-R51244	Carboniferous
Blattodea	<i>Euphyllodromia</i> sp.		Yes		Modern
	<i>Mastotermes darwiniensis</i> Froggatt, 1897		Yes		Modern
	<i>Megaloblatta</i> sp.		Yes		Modern
	<i>Periplaneta americana</i> (Linnaeus, 1758)	ESRF	Yes	MNHN EP 4339	Modern
	<i>Polyphaga aegyptiaca</i> (Linnaeus, 1758)		Yes		Modern
	<i>Protagonista</i> sp.		Yes		Modern
Mantodea	<i>Acanthops fuscifolia</i> Olivier, 1792		Yes		Modern
	<i>Amorphoscelis</i> sp.		Yes		Modern

	<i>Chaeteessa caudata</i> Saussure, 1871		Yes		Modern
	<i>Choeradodis</i> sp.		Yes		Modern
	<i>Hymenopus coronatus</i> Olivier, 1792		Yes		Modern
	<i>Metallyticus</i> sp.		Yes		Modern
	<i>Pnigomantis medioconstricta</i> Westwood, 1889		Yes		Modern
	<i>Stagmatoptera supplicaria</i> (Burmeister, 1838)	UP	Yes	MNHN EP 577	Modern
†Hypoperlida	† <i>Hypoperla elegans</i> Martynov, 1928		Yes	PIN 3353/415	Permian
Psocoptera	<i>Loensia fasciata</i> (Fabricius, 1787)		Yes		Modern
†Permopsocida	† <i>Psocorrhyncha burmitica</i> Huang et al., 2016		Yes	NIGP161473	Cretaceous
Thysanoptera	<i>Megalothrips delmasi</i> Bournier, 1956		Yes		Modern
Hemiptera	<i>Lyristes plebejus</i> (Scopoli, 1763)		Yes		Modern
	<i>Phrictus auromaculatus</i> Distant, 1905		Yes		Modern
	<i>Afrolidia boukokoensis</i> Nielson, 1992		Yes		Modern

	<i>Ptyelus flavescens</i> (Fabricius, 1794)		Yes		Modern
	<i>Cacopsylla mali</i> (Schmidberger, 1836)		Yes		Modern
	<i>Halyomorpha halys</i> Stål, 1855		Yes		Modern
Hymenoptera	<i>Xyela julii</i> Brébisson, 1818		?		Modern
	<i>Urocerus gigas</i> (Linnaeus, 1758)		?		Modern
Coleoptera	<i>Cerambyx cerdo</i> Linnaeus, 1758		Yes		Modern
	<i>Oryctes</i> sp.		Yes		Modern
	<i>Cupes raffrayi</i> Fairmaire, 1885	UP	Yes		Modern
Megaloptera	<i>Corydalis</i> sp.		Yes		Modern
Neuroptera	<i>Cymothales</i> sp.		Yes		Modern
	<i>Glyptobasis</i> sp.		Yes		Modern
	<i>Polystoechotes punctatus</i> Fabricius, 1793		Yes		Modern
Raphidioptera	<i>Fibla peyerimhoffi</i> (Navás, 1919)		Yes		Modern
Trichoptera	<i>Semblis phalaenoides</i> (Linnaeus, 1758)		Yes		Modern
	<i>Molanna albicans</i> (Zetterstedt, 1840)		Yes		Modern
	<i>Notidobia ciliaris</i> (Linnaeus, 1760)		Yes		Modern

	† <i>Necrotaulius proximus</i> Sukatsheva 1973		Yes		Trias
	<i>Philopotamus montanus</i> (Donovan, 1813)		Yes		Modern
Lepidoptera	<i>Korscheltellus lupulinus</i> (Linnaeus, 1758)		Yes		Modern
	<i>Micropterix aureatella</i> (Scopoli, 1763)		Yes		Modern
	<i>Agathiphaga queenslandensis</i> Dumbleton, 1952		Yes		Modern
Mecoptera	<i>Harpobittacus australis</i> Klug, 1838		Yes		Modern
	<i>Bittacus chilensis</i> Klug, 1838		Yes		Modern
	<i>Panorpa germanica</i> Linnaeus, 1758		Yes		Modern
Diptera	<i>Tipula maxima</i> Poda, 1761		?		Modern
	<i>Tabanus autumnalis</i> Linnaeus 1761		?		Modern

Imaging:

the nine specimens, for whom we report here 3D observations, were imaged under X-ray. XMT was performed according to the protocols reported in the literature (Boistel *et al.*, 2011; Lauridsen *et al.*, 2011; Zanette *et al.*, 2013). At the ‘PLATeforme INstrumentale d'Analyses’ (PLATINA, IC2MP) of the University of Poitiers (France), we used a microtomograph RX solutions EasyTom XL Duo, using a Hamamatsu nanofocus 160 kV 8W source and a Varian-Paxscan 2520DX CsI detector. At the microtomograph of the MNHN, platform ASTRX, we

used a v|tome|x L 240-180, GE Sensing & Inspection Technologies phoenix x|ray. At the ESRF (Grenoble), we used the ID17 line. To be scanned, each forewing was most often separated from the body and placed in a plastic tube, parallel at the beam of CT-scan. For each specimen, the image acquisition parameters are listed in Suppl. Table 2.

3D volume rendering (Isosurface) was used to visualize the sub-set of selected voxels of the wings structure in AVIZO (FEI, Merignac, France, <https://www.fei.com/software/amiraavizo/>). This was performed using a manual segmentation tools with a limitation of level of grey (Supplementary Figs 1-2). The segmentation process resulted for each dataset in a ‘label’ dataset with the same dimensions as the corresponding slice. The microtomography analyses show the wing membrane, the ribs and thoracic basal sclerites. For convenience, we have chosen to reproduce in 3D models the ribs, basivenale bullae, and main veins. Most of the crossveins that were visible on tomograms were not essential for the study; therefore, they generally do not appear. In order to complete the 3D modeling, each specimen was observed under a binocular microscope, model Olympus SZX9, with a target PLAPO 1X DF-2 and Nikon SMZ25 Microscope. The wing venations were photographed using a Nikon D800 digital camera and a Nikon SMZ25 Microscope, assembled with Helicon Focus (multifocus method) and modified using Adobe Photoshop CS and DxO PhotoLab softwares.

Supplementary table 2. List of taxa and parameters for CT-scan segmentation

Taxon Collection number	Accelerating voltage (kV)	Intensity (μA)	Numerisation angle (°)	Number projections	Voxel size (μm)
MNHN EP578	70	27	360	1440	4.44
MNHN EO PHAS 1083	65	230	360	2300	32.08

MNHN EO CAELIF 3000	80	230	360	1700	14.10
MNHN EO ENSIF 3080	120	110	360	1300	18.44
MNHN EP 577	70	27	360	1440	6.57
MNHN EP 4339	Not given				3.04
MNHN EP 4340	Not given				3.04
MNHN EP 4342	Not given				1.47
<i>Aeshna isoceles</i>	70	33	360	1184	3.45
<i>Cupes raffrayi</i>	80	26	360	2976	2.73

Discussion

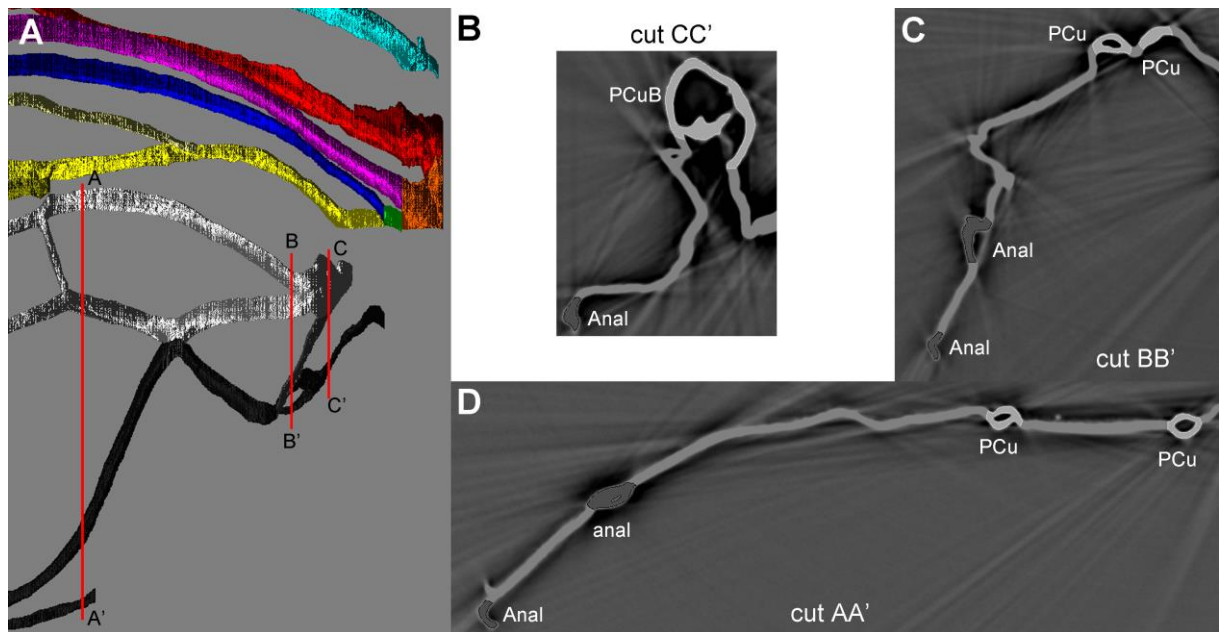
Homologies of wing veins in the extant Orthoptera:

Desutter-Grandcolas et al. (2017), in a morphological analysis of the venation of the Orthoptera:

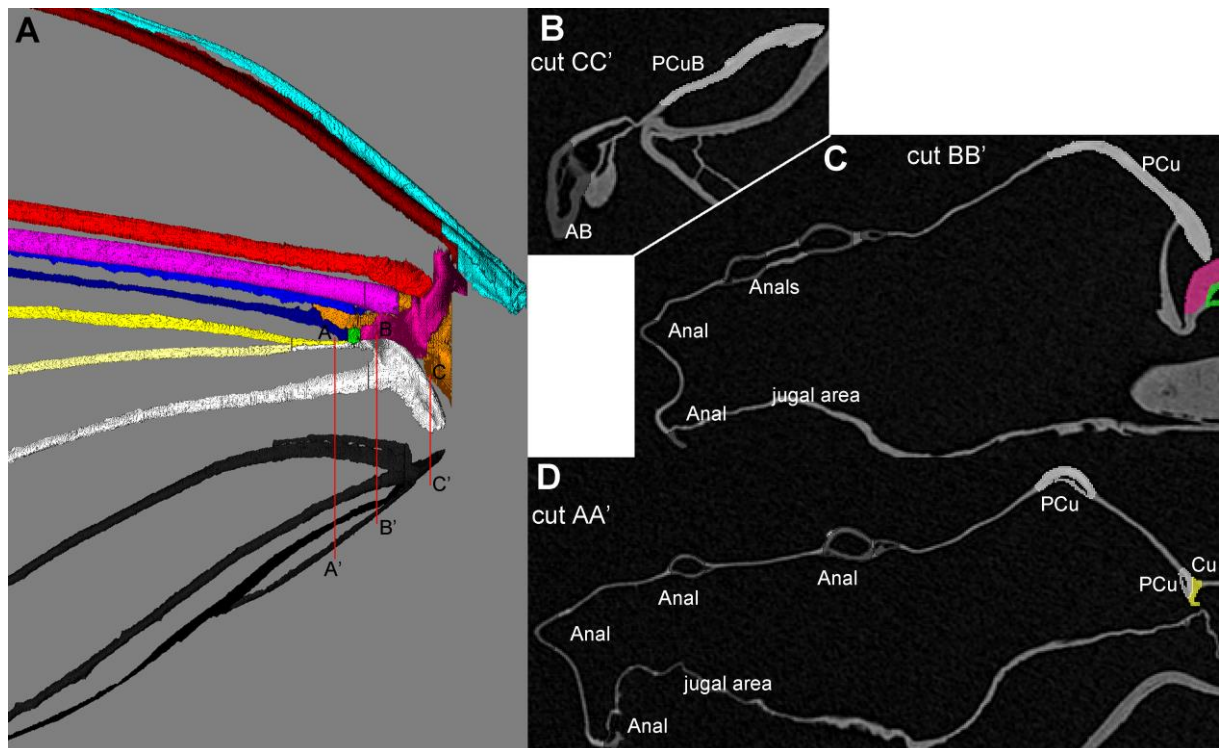
Ensifera, considered that the singing file of the Grylloidea and Tettigonioidae is on the first anal vein, while it is on the most posterior branch of the vein CuP in the Gryllotalpoidea and Hagloidea. The vein they named first anal (or A1) is in fact the most anterior branch of PCu vein. In this particular case, it does not change much in the evolutionary consequences of the study of Desutter-Grandcolas et al. (2017), but could be more important for comparisons with the situation in other taxa, especially fossils, e.g, the Permostridulidae and the fossil taxa currently in Hagloidea (Gorochoff, 1995; Béthoux et al., 2003).

References

- Béthoux, O., Nel, A., Lapeyrie, J., Gand, G. and Galtier, J. 2003. The Permostridulidae, a new enigmatic insect family from the Upper Permian of France. *European Journal of Entomology*, 100 (4): 581-585.
- Boistel, R., Herrel, A., Lebrun, R., Daghfous, G., Tafforeau, P., Losos, J.B. & Vanhooydonck, B. (2011) Shake rattle and roll: the bony labyrinth and aerial descent in squamates. *Integrative Comparative Biology*, **56**, 957–968.
- Desutter-Grandcolas, L., Jacquelin, L., Hugel, S., Boistel, R., Garrouste, R., Henrotay, M., Warren, B.H., Chintauan-Marquier, I.C., Nel, P., Grandcolas, P. and Nel, A. 2017. 3-D imaging reveals four extraordinary cases of convergent evolution of acoustic communication in crickets and allies (Insecta). *Scientific Reports*, 7(1) (7099): 1-8.
- Gorochoy, A.V. 1995. Sistema i evolyutsiya pryamokrylykh podotryada Ensifera (Orthoptera) [System and Evolution of the suborder Ensifera (Orthoptera).] Parts 1 and 2. *Trudy Zoologicheskogo Instituta* [Russian Academy of Sciences, Proceedings of the Zoological Institute], St.-Petersburg, 260: 3-224 + 261: 3-212. [in Russian]
- Lauridsen, H., Hansen, K., Wang, T., Agger, P., Andersen, J.L., Knudsen, P.S., Rasmussen, A.S., Uhrenholt, L. & Pedersen, M. (2011) Inside out: modern imaging techniques to reveal animal anatomy. *PLoS ONE*, **6**, e17879.
- Zanette, I., Daghfous, G., Weitkamp, T., Gillet, B., Adriaens, D., Langer, M., Cloetens, P., Helfen, L., Bravin, A., Peyrin, F., Baumbach, T., Dischler, J.-M., Van Loo, D., Praet, T., Poirier-Quinot, M. & Boistel, R. (2013) Looking inside marine organisms with magnetic resonance and X-ray imaging. pp. 122–184. In: Reynaud, E.G. (ed.) *Imaging marine life*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.



Supplementary Fig. 1. Plecoptera *Perla marginata* (Panzer, 1799), forewing base, 3D reconstruction, dorsal view, with three cuts showing the course of the postcubital and anal veins and bullae. A, reconstruction; B-D, cuts. (copyright Thomas Schubnel).



Supplementary Fig. 2. Mantodea *Stagmatoptera supplicaria* (Stoll, 1813), forewing base, 3D reconstruction, dorsal view, with three cuts showing the course of the postcubital and anal veins and bullae. A, reconstruction; B-D, cuts. (copyright Thomas Schubnel).