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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

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

32

33 Introduction

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

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

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

53 Among those conflicts (Table 1), the vein between the cubital and anal complexes has been
54 identified either as a vein named postcubital or empusal vein (PCu or E), independent of the
55 cubital or anal stems (e.g., Lameere, 1922; Snodgrass, 1935; Hamilton, 1972; Emeljanov,
56 1977), or as the first anal vein attached to the anal stem (e.g., Comstock & Needham, 1898,
57 1899; Wootton, 1979; Kukalová-Peck, 1992). Authors brought various evidences using
58 different criteria to refine their homology hypotheses (e.g., tracheae, axillary sclerites, folds of
59 wings). They also recognized that all of these criteria, when used individually, have defects
60 (Hamilton, 1972) so that no hypothesis has received unanimous support (e.g., Matsuda, 1970
61 *contra* Wootton, 1979). Thus, the exact nature and the existence of an independent postcubital
62 vein between the cubital and anal veins remain unsolved and hinder sound homology
63 hypotheses at the largest insect scale.

64 Consequently, several venation groundplans exist and are currently used to describe insect
65 venation. Most authors use the venation terminology proposed by Comstock & Comstock
66 (1895), modified by Comstock & Needham (1898, 1899), and summarized by Comstock
67 (1918). Kukalová-Peck (1991) proposed a second groundplan also currently used. A third
68 proposal of groundplan with a further PCu vein between Cu and A, originally proposed by
69 Lameere (1922) (PCu as ‘Pénultième’ vein) and partly followed by Forbes (1943) (PCu as Plical
70 vein or Pl), was supported by Snodgrass (1935), Matsuda (1970), Emeljanov (1977), and
71 Brodsky (1994). It was followed incidentally for Trichoptera and Lepidoptera (Ivanov, 1985,
72 1995), more frequently for Hemiptera (e.g., Franielczyk-Pietyra & Wegierek, 2019), and very
73 recently for Blattodea (Li *et al.*, 2018). The PCu vein corresponds to the anterior anal vein (AA)
74 of Kukalová-Peck (1991) and to the first anal branch of Comstock (1918), as already noticed
75 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		

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

84

85 Material and methods

86 Wing venation terminology and color code:

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

92 Criteria for venation homology:

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

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

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

115 **Taxonomic sampling:**

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

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

140 **Ancestral state reconstruction:**

141 We used the parsimony method with Winclada v.1.00.08 (Nixon, 2002) to reconstruct the
142 ancestral states for the character ‘presence of a PCu’ in insects. We used a simplified tree
143 derived from the phylogeny of Wipfler *et al.* (2019: Fig. 1), with ‘Protelytroptera’ as sister
144 group of Dermaptera (Béthoux *et al.*, 2016), Palaeodictyoptera within Palaeoptera,
145 Hypoperlida as sister group of crown Acercaria (Prokop *et al.*, 2017) and Permopsocida as sister
146 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).

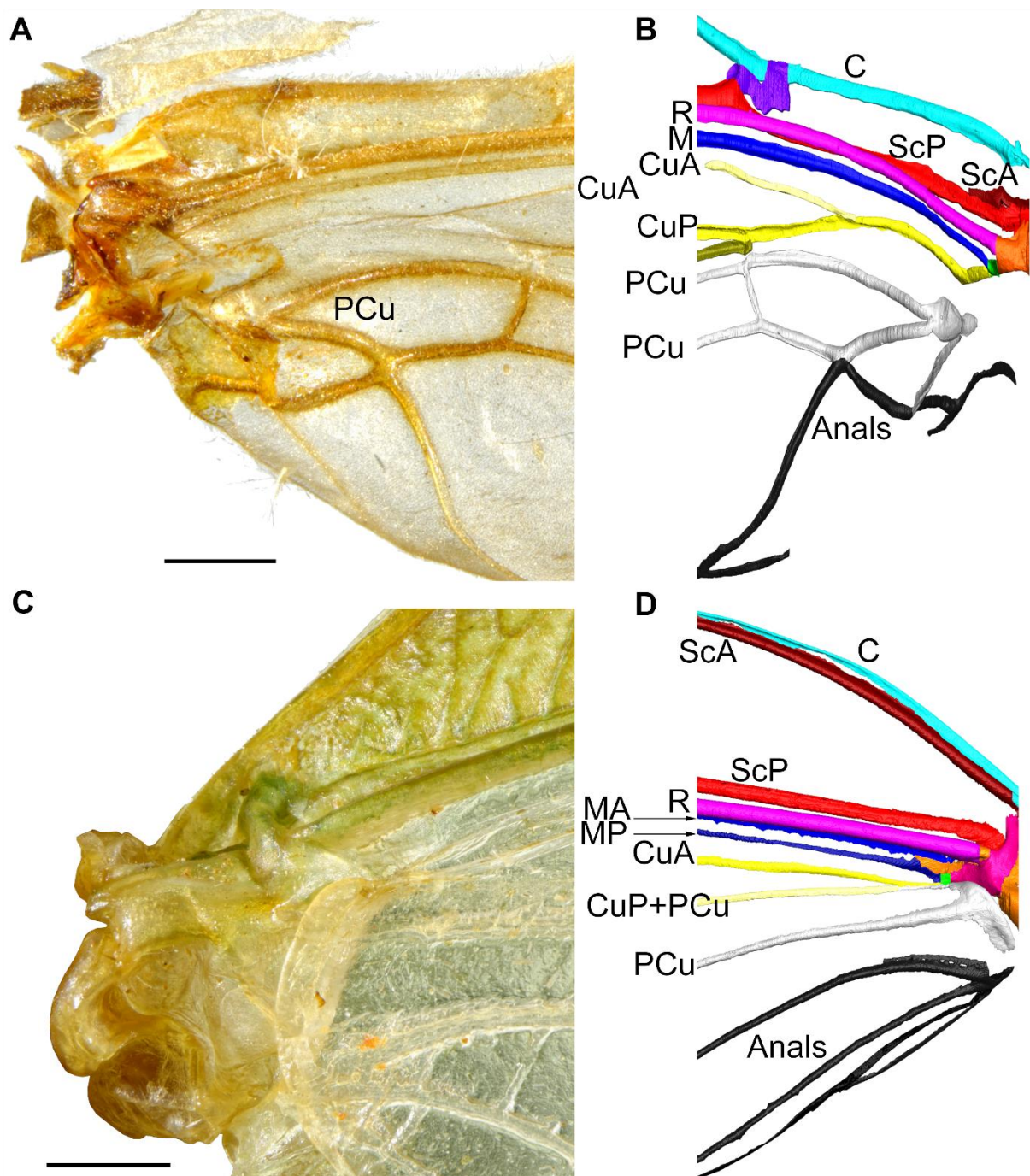


Fig. 1 : Forewing bases, photographs and 3D reconstructions, dorsal views. (A-B) Plecoptera *Perla marginata* (Panzer, 1799); (C-D) Mantodea *Stagmatoptera supplicaria* (Stoll, 1813). (copyright Thomas Schubnel). Scale bars = 1 mm.

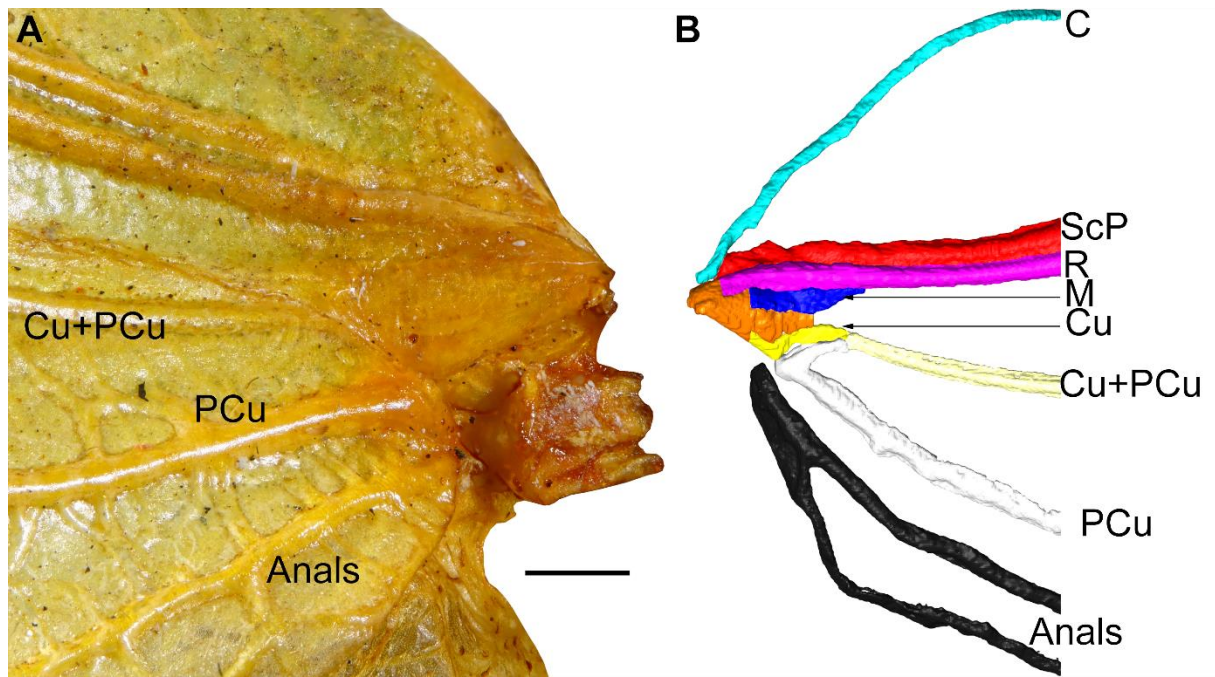


Fig. 2 : Forewing base, dorsal views. Phasmatodea *Phasma gigas* (Linnaeus, 1758). A, photograph; B, 3D reconstructions. (copyright Thomas Schubnel). Scale bars = 1 mm.

153 Results and Discussion

154 Bullae vs external sclerites:

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

161 Identification of the PCu from its characteristic bulla:

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

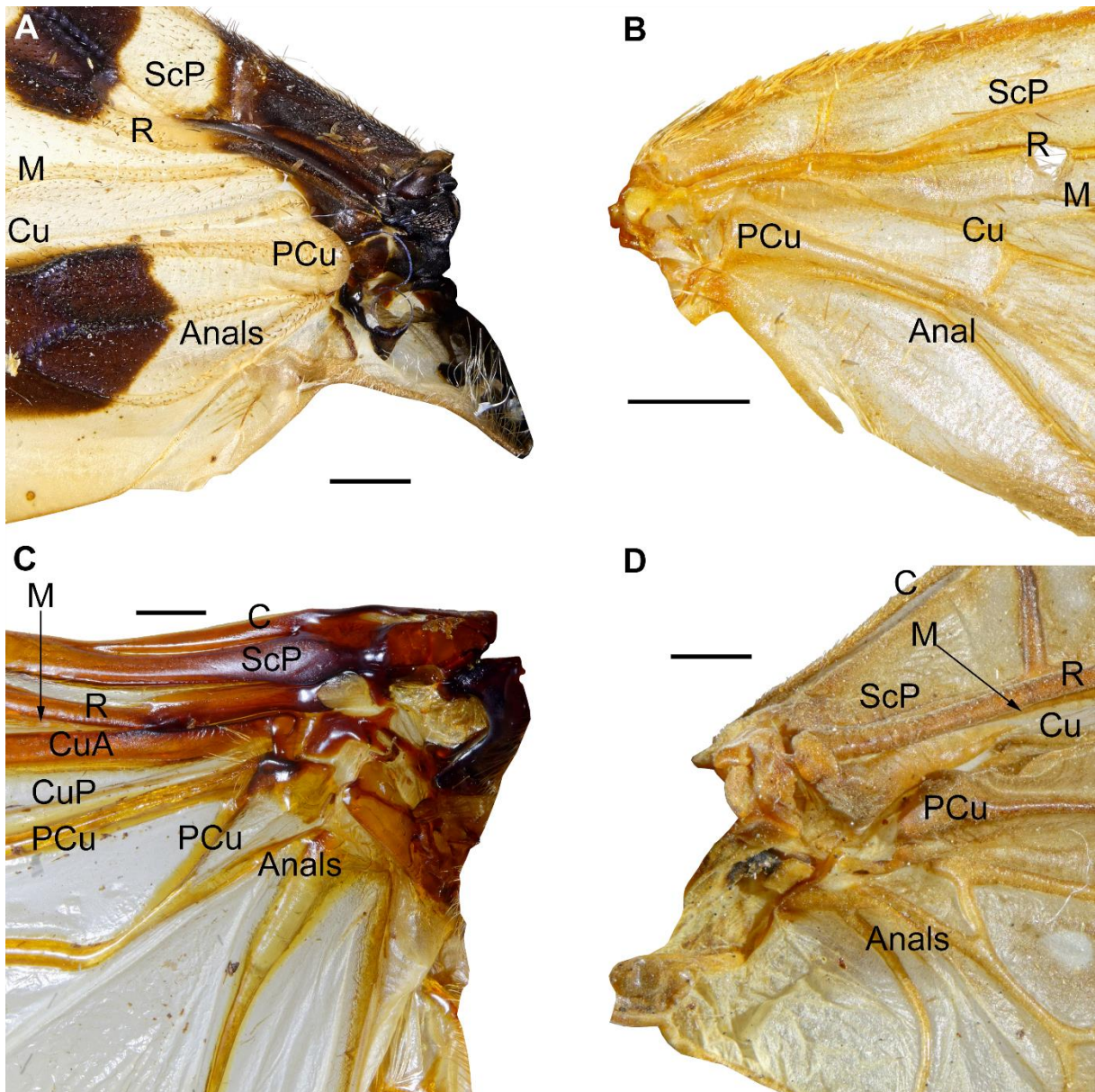


Fig. 3 : Forewing bases, photographs, dorsal views. A, *Trichoptera Semblis phalaenoides* (Linnaeus, 1758); B, *Lepidoptera Korscheltellus lupulinus* (Linnaeus, 1758); C, *Coleoptera Oryctes* sp.; D, *Megaloptera Corydalus* sp.; (copyright Thomas Schubnel). Scale bars = 1mm.

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

170 **Presence of the PCu in most winged insects:**

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

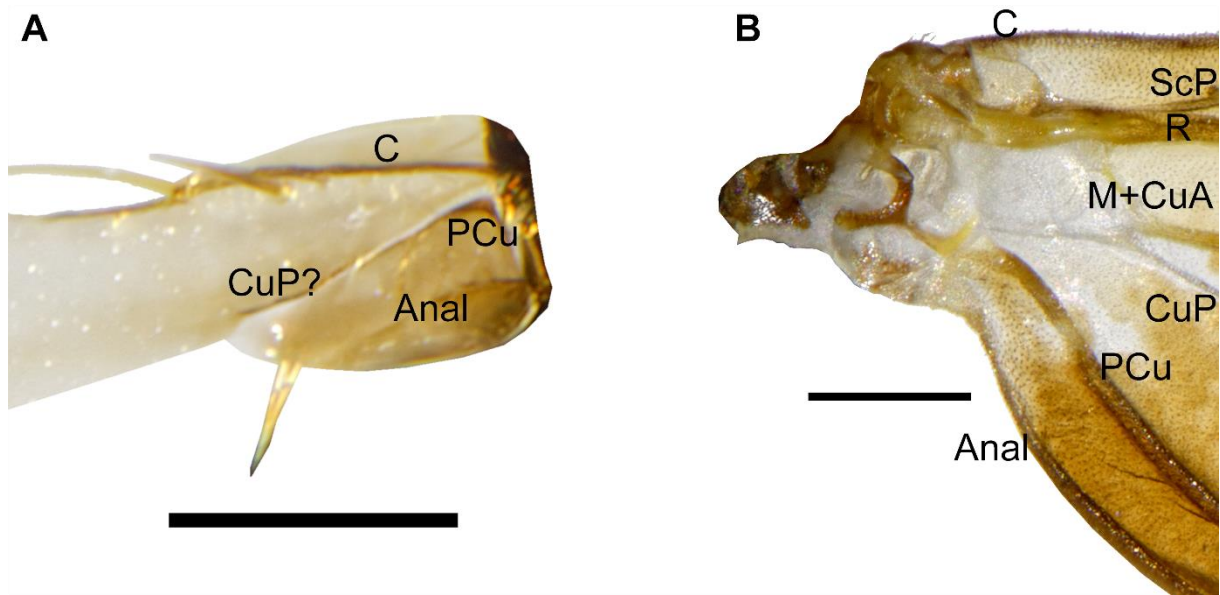


Fig. 5 : Forewing bases, photographs, dorsal views. A, Psocodea: Liposcelididae *Loensia fasciata* (Fabricius, 1787); B, Thysanoptera: Phaeothripidae *Megalothrips delmasi* Bournier, 1956. (copyright Thomas Schubnel). Scale bars = 0.25 mm.

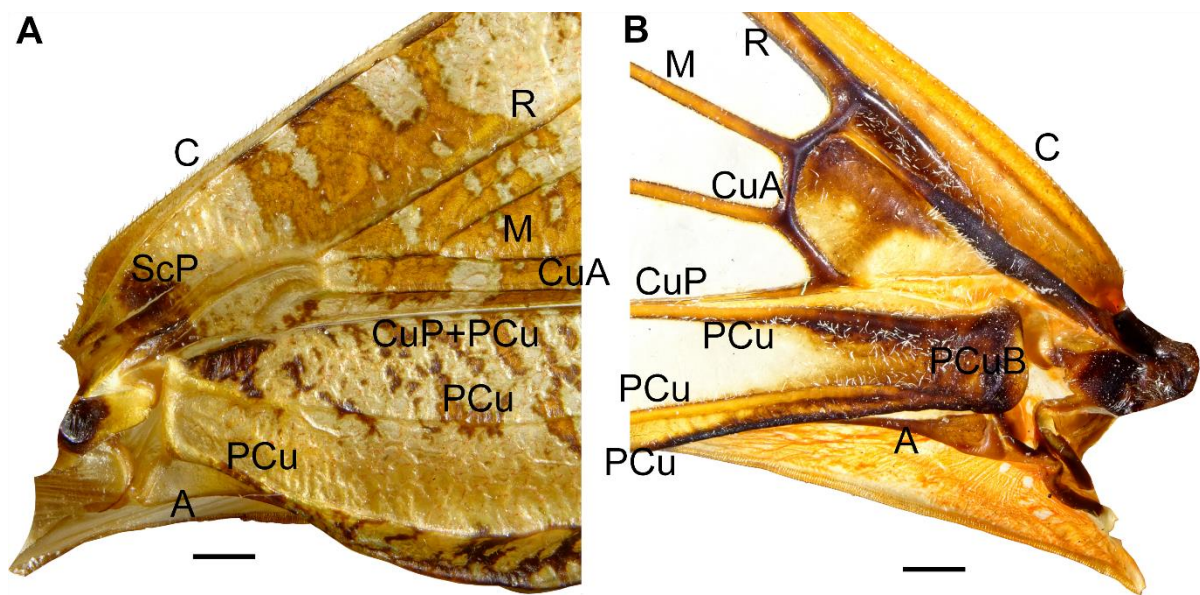


Fig. 4 : Forewing bases of Hemiptera, photographs, dorsal views. A, Fulgoridae *Phricтус auromaculatus* Distant, 1905; B, Cicadidae *Lyristes plebejus* (Scopoli, 1763). (copyright Thomas Schubnel). Scale bars = 1 mm.

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: Panorpidae, 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.

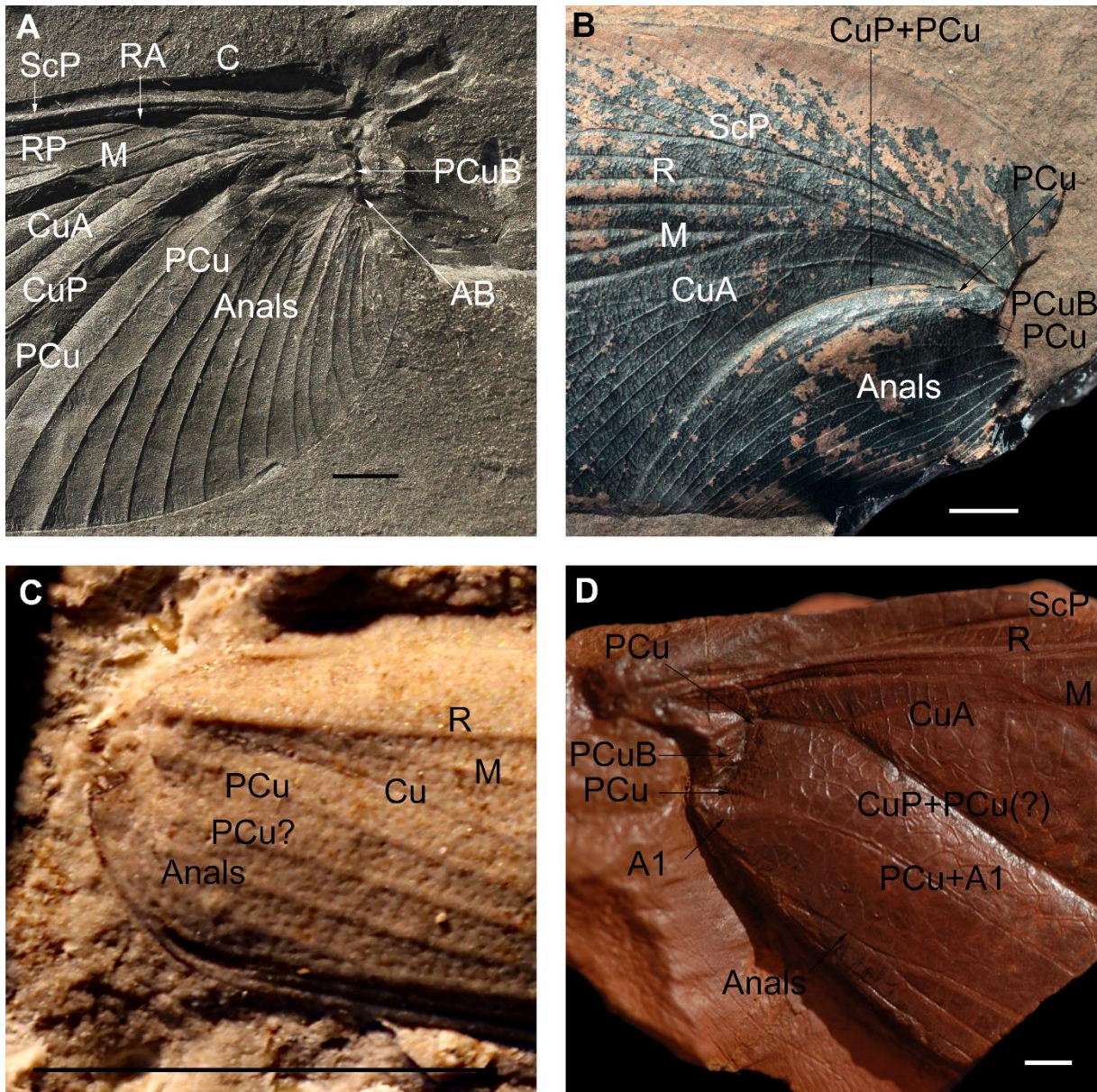


Fig. 6 : Wing bases of Paleozoic insects. A, †*Palaeodictyoptera Dunbaria quinquefasciata* (Martynov, 1940), hindwing, PIN No. 1631/311; B, *Dictyoptera Phyloblatta gaudryi* Agnus 1903, forewing, MNHN-F-R51244; C, †‘*Protelytroptera*’ *Protelytron furcatum* Carpenter, 1939, Paratype MCZ 3885; D, †*Paoliidae*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

182 3C), dobsonflies (Megaloptera: Corydalidae, Sialidae) (Fig. 3D), snakeflies (Raphidioptera),

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

184 We also identified the PCu bulla in the following fossils: a “six-winged insects”

185 (Palaeodictyoptera) (Fig. 6A), stem Dictyoptera (roachoids) (Fig 6B), Paoliidae (Fig. 6D),

186 Archaeorthoptera, Hypoperlida and Permopsocida. Extant Dermaptera have a much reduced

187 forewing venation that is hard to interpret but it is not the case of their putative stem group, the

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

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

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

207 **Taxa with unclear results:**

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

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

222 **Ancestral state reconstruction – origin of the PCu:**

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

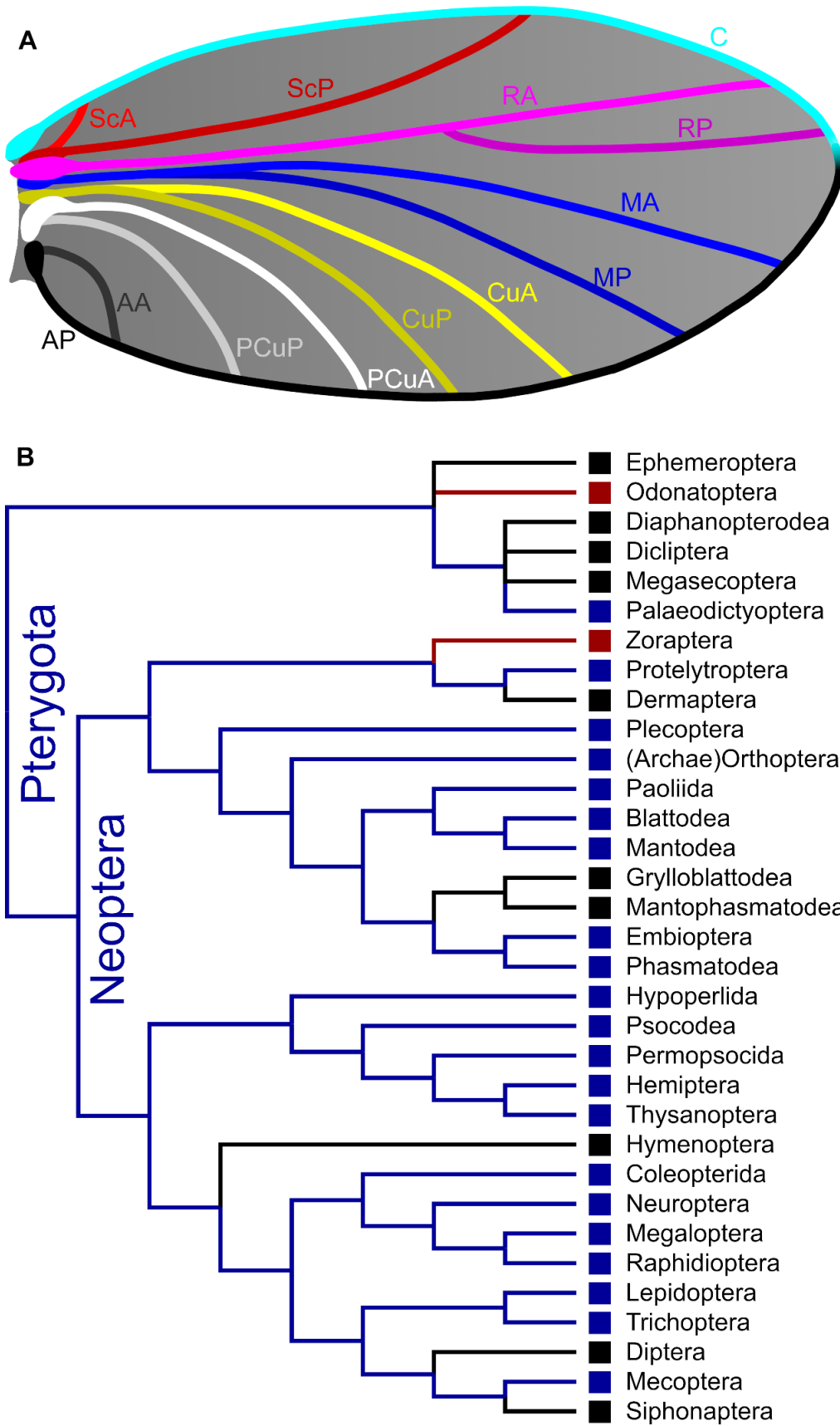


Fig. 7 : A, Proposed general pattern of insect wing venation with PCu veins. Posterior branches are in darker colors than anterior branches. B, Phylogenetic inference in parsimony of the presence of the PCu vein in Pterygota. Clades with PCu are in dark blue, those without are in red, and clades where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

235 **The PCu and its implications:**

236 Our results support Snodgrass (1935), Emeljanov (1977), Brodsky (1994), and Li *et al.* (2018)
237 about the existence of a postcubital vein, independent of the cubital and the anal veins. These
238 three veins emerge from three independent bullae. We go further, however, demonstrating that
239 a majority of orders have a PCu and that the situation is even more complex than supposed by
240 these authors. They generally considered that the PCu is not fused or closely adjacent to the
241 branch(es) of the cubital system. Emeljanov (1977) and Brodsky (1994) thought that the PCu
242 is a simple vein, posterior to CuP, in all insects. Li *et al.* (2018) stated that the anal veins of
243 Comstock–Needham should be interpreted as two independent main veins, their PCu and
244 ‘vannal’ veins, on the basis of a supposed correspondence between the trachea and the veins in
245 Blattodea. In fact, in many neopteran orders, the PCu is forked with its anterior branch
246 appressed or partly fused to the CuP [e.g., Dictyoptera, for which Kukalová-Peck (1992)
247 indicated a fusion between her ‘CuP’ and ‘AA1’ (= PCu)].
248 The presence of the PCu also implicates that the anal area is smaller than previously suggested,
249 which has implication on the evolution of flight and notably on the ‘umbrella effect’ (*sensu*
250 Wootton, 1992: 131): “the soft vannus is supported by a series of unbranched anal and jugal
251 veins radiating from the base.” In Holometabola, the anal area is often reduced to only one vein.
252 In Plecoptera, the anal area is strongly reduced in some species (e.g. *Filchneria olgae*
253 (McLachlan, 1875)) and strongly developed with numerous veins associated to a reduced post-
254 cubital area in others (e.g. *Eusthenia spectabilis* Westwood, 1832) (Fig. 8). In some groups,
255 such as Blattodea and Palaeodictyoptera, in which the ‘anal area’ is considered as very
256 developed, the existence of the PCu vein modifies the interpretation/identities/homologies of
257 ‘anal area’ veins. The Palaeodictyoptera have numerous anal and post-cubital veins, while
258 Blattodea have few post-cubital veins and several anal veins. So, the recognition of the PCu as
259 an independent vein sheds a new light on some cases of evolutionary convergences for the
260 ‘umbrella effect’ (Desutter-Grandcolas *et al.*, 2005; Wootton, 1992).

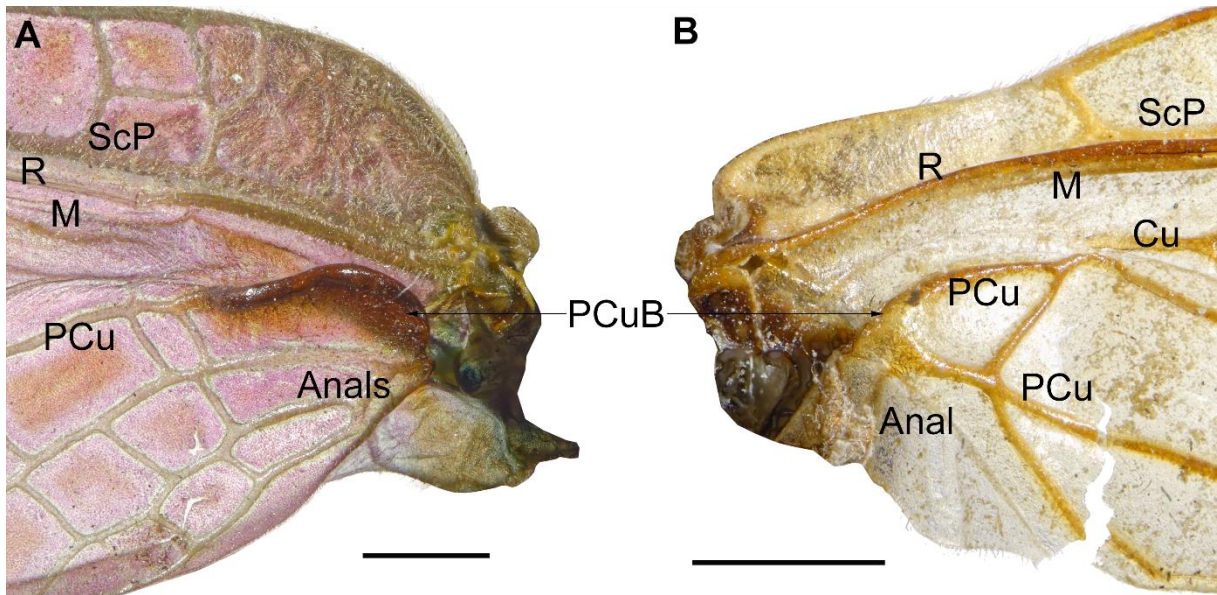


Fig. 8 : Forewing bases, photographs, dorsal views. Plecoptera. A, Eustheniidae *Eusthenia spectabilis* Westwood, 1832, Perlodidae *Filchneria olgae* (McLachlan, 1875). (copyright Thomas Schubnel). Scale bars = 1 mm.

261 **The specific case of Phasmatodea:**

262 The extant Phasmatodea show an unusual forewing venation in which the main veins are
 263 difficult to recognize. Ragge (1955) proposed a pattern based on a putative correspondence
 264 between veins and trachea, that was questioned by Nel & Delfosse (2011) and Wang et al.
 265 (2014) on the basis of the study of the venation of Mesozoic taxa currently attributed to the
 266 stem group of Phasmatodea. These last authors considered that these fossils had venations of
 267 archaeorthopteran type (sensu Béthoux & Nel, 2002), but this last pattern was not recovered in
 268 extant stick insects. After our CT reconstructions, extant stick insects have a median vein
 269 extremely reduced and appressed to the radial vein and a simple vein Cu, completely separated
 270 from the median and radial veins, either only present at the extreme wing base and immediately
 271 ending into the anterior branch of PCu (Fig. 2) (e.g., *Phasma gigas* (Linnaeus, 1758)), or
 272 independent from PCu (e.g., *Tropidoderus childrenii* Gray, 1833). The PCu structure consists
 273 in two clearly convex veins, emerging from a bulla in domed structure, independent of cubital
 274 and anal bullae. Previous authors considered these two PCu veins as cubital veins (Ragge,
 275 1955), or anal veins (Nel & Delfosse, 2011). Thus the extant stick insects have an extremely
 276 particular forewing venation unique among all the Pterygota, with the median and cubital veins

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

281

282 Conclusion

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

300

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314

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Supplementary material

Material and methods

Abbreviations of institutions: MNHN (Muséum national d'Histoire naturelle, Paris); NIGP (Nanjing Institute of Geology and Palaeontology, 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 ($^{\circ}$)	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

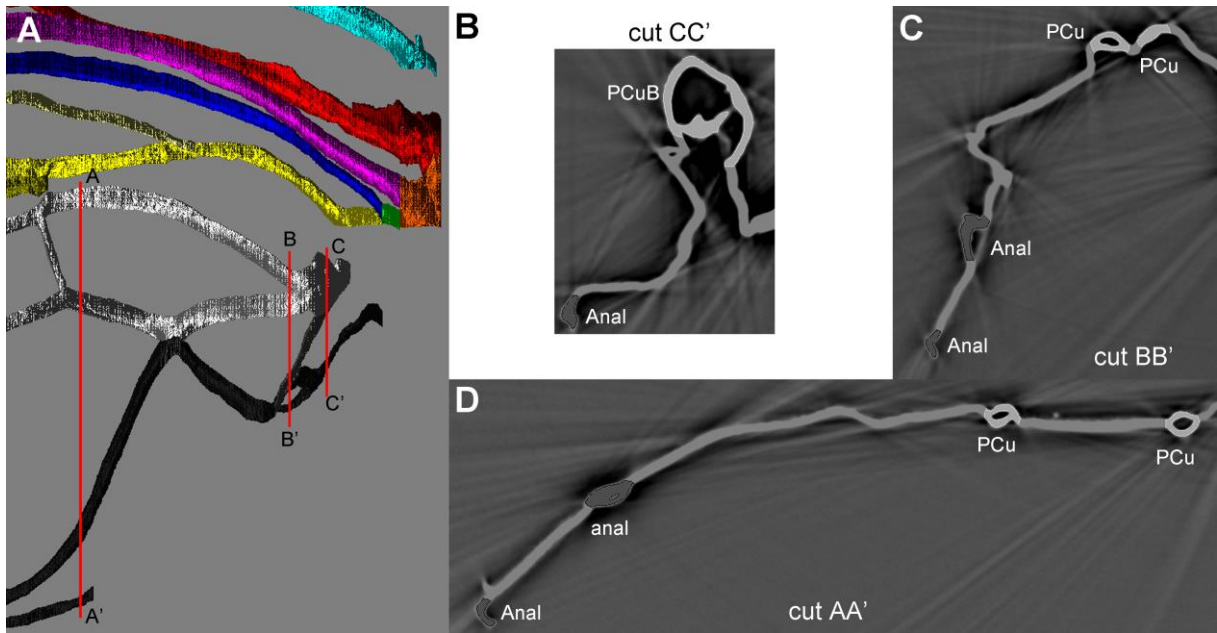
Discussion

Homologies of wing veins in the extant Orthoptera:

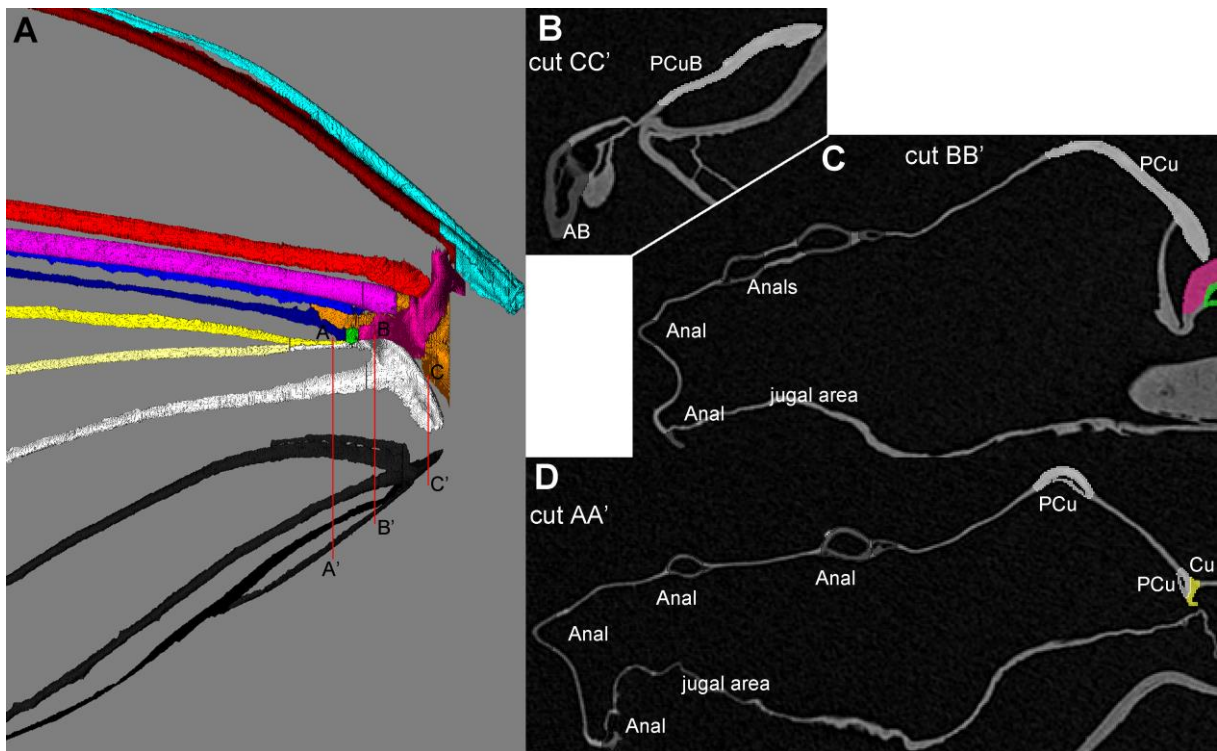
Desutter-Grandcolas et al. (2017), in a morphological analysis of the venation of the Orthoptera: Ensifera, considered that the singing file of the Grylloidea and Tettigonioidea is on the first anal vein, while it is on the most posterior branch of the vein CuP in the Gryllotalpoidea and Hagloidea. The 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 (Gorochov, 1995; Béthoux et al., 2003).

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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).