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# Life habits and evolutionary biology of new two-winged long-proboscid scorpionflies from mid-Cretaceous Myanmar amber

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Long-proboscid scorpionflies are enigmatic, mid-Mesozoic insects associated with gymnosperm pollination. One major lineage, Aneuretopsychina, consists of four families plus two haustellate clades, Diptera and Siphonaptera. One clade, Pseudopolycentropodidae, from mid-Cretaceous Myanmar amber, contains *Parapolycentropus*. Here, we newly establish *Dualula*, assigned to Dualulidae, constituting the fifth lineage. *Parapolycentropus* and *Dualula* lineages are small, two-winged, with unique siphonate mouthparts for imbibing pollination drops. A cibarial pump provides siphonal food inflow; in *Dualula*, the siphon base surrounds a hypopharynx housing a small, valved pump constricted to a narrow salivary duct supplying outgoing enzymes for food fluidization. Indirect evidence links long-proboscid mouthpart structure with contemporaneous tubulate ovulate organs. Direct evidence of gymnospermous *Cycadopites* pollen is associated with one *Parapolycentropus* specimen. *Parapolycentropus* and *Dualula* exhibit hind-wing reduction that would precede haltere formation, likely caused by *Ultrabithorax*. Distinctive, male Aneuretopsychina genitalia are evident from specimens in copulo, supplemented by mixed-sex individuals of likely male mating swarms.

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Long-proboscid scorpionflies (Mecoptera) have a long evolutionary history of interacting with plants in Eurasia beginning during the late Permian and ending in the mid Cretaceous. The earliest scorpionfly lineage with long-proboscid mouthparts, defined by a projecting siphon, was Nedubroviidae from late Permian Russia[1,2]. Taxa within this small-bodied lineage survived the Permian–Triassic ecological crisis into the ensuing Triassic[2,3], supplemented by two lineages of long-proboscid scorpionflies, Mesopsychidae and Pseudopolycentropodidae. During the mid Mesozoic, with proliferation of additional taxa from three other insect orders, there minimally were 13 independent originations of long-proboscid mouthparts[4]. These long-proboscid groups included Mecoptera[5–7] (scorpionflies, three originations from this study), Neuroptera[8,9] (lacewings, three originations), Lepidoptera[10,11] (moths and butterflies, one origination), and Diptera[12,13] (true flies, six originations).

For Mecoptera, all long proboscid taxa historically were contained within the presumably monophyletic, latest Paleozoic to mid-Mesozoic lineage, Aneuretopsychina[14], which comprised four families—Nedubroviidae[2], Mesopsychidae[3], Pseudopolycentropodidae[15–17], and Aneuretopsychidae[14]. The Nedubroviidae consisted of *Nedubrovidia shcherbakovi* and three other congeneric species from Late Permian European Russia at ca. 254 Ma[2]. The last lineage, Pseudopolycentropodidae, is documented from several mid-Mesozoic deposits, including *Parapolycentropus burmiticus* and *P. paraburmiticus*[16,17] from mid-Cretaceous Myanmar (Burmese) amber at 99 Ma[18]. Aneuretopsychina had two intervals of diversification—an earlier, modest resurgence during the latter Triassic, and a greater speciation interval from the Middle Jurassic to Early Cretaceous. Understanding of Aneuretopsychina biology has increased greatly from examination of compression deposit occurrences[2,3,6,7,19,20], to a recent focus on late appearing lineages from Myanmar amber[16,17,21]. Examination of these amber taxa soon before extinction of Aneuretopsychina now can provide more finely resolved details of the life habits and evolutionary biology of this bizarre[17] group of insects.

In this contribution, we provide long-proboscid scorpionfly data from Middle Jurassic compression deposits of Northeastern China, and mid-Cretaceous amber from Northern Myanmar. These taxa are placed into a phylogenetic context within lineages of extant and extinct Mecoptera that are linked phylogenetically to Amphiesmenoptera and Neuroptera outgroups, as well as ingroups including seven basal lineages of Diptera and Siphonaptera. The new family that we establish constitutes a modification of siphonate, pseudopolycentropodid-type mouthparts not documented in any other, known, long-proboscid group. A sufficiently well preserved number of insect specimens have been marshalled to provide evidence for documenting transformation of the mecopteran hind wing into a haltere-like structure by reference to the *Ultrabithorax* homeotic gene system in *Drosophila*. We document structurally well-preserved male genitalia from compression Mesopsychidae and Pseudopolycentropodidae that are compared to the amber taxa, the latter including an in copulo pair, revealing stereotyped patterns of scorpionfly genitalia structure during the mid Mesozoic. Similarly, from three amber pieces containing mixed sex and species congregations of two *Parapolycentropus* species, we describe evidence for lekking swarms. Our documentation of a new Pseudopolycentropodidae lineage establishes a new, long-proboscid family that, together with its closely related sister-taxon, possesses a unique, new mouthpart type that allows comparisons to other extinct and extant long-proboscid morphologies. From a variety of indirect and direct evidence, we provide an explicit explanation of the feeding mechanisms of these taxa, and their association with gymnosperm hosts based on mouthpart structure, host-plant ovulate organ morphology, and adjacent pollen. Our multifaceted study should enlarge knowledge of long-proboscid scorpionfly ecology and their life habits from the deep past.

## Results

**Phylogenetic analysis**. We conducted a phylogenetic analysis to clarify the taxonomic position of new family and understand the relationships of long-proboscid clade (Aneuretopsychina), as well as Mecoptera in general. This analysis included two relevant representatives of Neuroptera and Amphiesmenoptera as outgroups, and Siphonaptera and seven early appearing, basal lineages of Diptera as ingroups. The input data consisted of 27 major lineages of extinct and extant Mecoptera that sampled a wide diversity of body form (Supplementary Data 1 and 2). A full complement of 51 morphologic characters coding 37 total taxa represented head, wing, leg, thorax and abdominal features (Supplementary Note 1).

A maximum parsimony analysis yielded 93 most parsimonious trees. The strict consensus result (Fig. 1a) has a tree length of 159 steps, consistency index (CI) of 0.34 and retention index (RI) of 0.73. Morphological characters were optimized with parsimony on all most-parsimonious trees, showing only unambiguous changes. We chose the twenty-fourth generated tree (Fig. 1b) as the most suitable tree based on a summary of pre-existing phylogenetic conclusions from several previous analyses of Panorpoidea sensu stricto, including Dinopanorpidae, Orthophlebiidae, Panorpidae, Panorpodidae[22] and basal Diptera[23] (Supplementary Fig. 1). Bootstrap values are shown in Fig. 1.

The phylogenetic analysis provided five important results (Fig. 1). First, Mecoptera are a paraphyletic group and Thaumatomeropidae and Kaltanidae are basalmost taxa. Second, Meropeidae and Eomeropidae are more basal than other extant and extinct families of Mecoptera. Third, long-proboscid Aneuretopsychina are a paraphyletic group, with *Parapolycentropus* + Dualulidae having closer affinities to basal Diptera + Siphonaptera. However, the unknown mouthpart structure of Liassophilidae and Permotanyderidae calls into question the phylogenetic status of Aneuretopsychina, requiring further investigation with additional, well-preserved fossils from these lineages that reveal mouthpart structure. Fourth, Aneuretopsychina, including Pseudopolycentropodidae, Aneuretopsychidae, Mesopsychidae, and Nedubroviidae, and possible long-proboscid Liassophilidae and Permotanyderidae, are a sister-clade to a (*Parapolycentropus* + Dualulidae) + (basal Diptera + Siphonaptera) clade. Fifth, Aneuretopsychina likely were phenetically similar to the immediate ancestor of Diptera, particularly as the *Parapolycentropus* + Dualulidae lineage exhibits close affinities to basal dipteran taxa and Siphonaptera.

### Systematic palaeontology.

Order Mecoptera Packard, 1886
Suborder Aneuretopsychina Rasnitsyn and Kozlov, 1990.
Family Dualulidae Lin, Shih, Labandeira and Ren, fam. nov.

**Type genus**. *Dualula* Lin, Shih, Labandeira and Ren gen. nov. (Figs. 2 and 3; head and mouthparts reconstructed in Fig. 4).

**Diagnosis**. Body size small, length ca. 7.6–8.4 mm (excluding antennae and proboscis). Head triangular in dorsal view, with long, narrow proboscis. Antennae filiform, slender; shorter than proboscis. Compound eyes large,

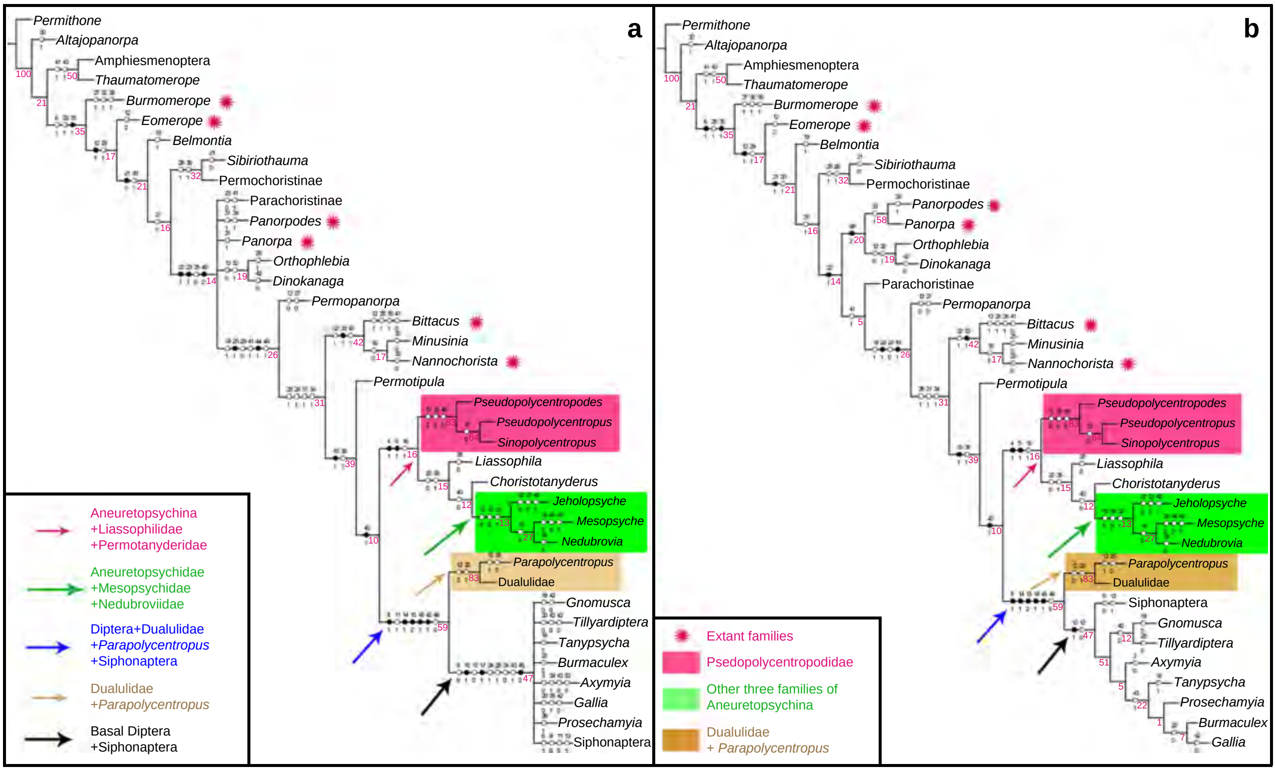

**Fig. 1** Results of the phylogenetic analysis by NONA. **a** Strict consensus tree of 93 maximum parsimony trees from NONA. **b** The twenty-fourth maximum parsimony tree from the NONA analysis. Open circles are plesiomorphic characters; solid black circles are apomorphic characters. The numbers above branches are characters; numbers below the branches are character states; and magenta numbers below the branches are bootstrap values in **a** and **b**. Colored arrows refer to the following clades or lineages: magenta = Aneuretopsychina sensu lato; green = Aneuretopsychidae + (Mesopsychidae + Nedubroviidae); blue = (*Parapolycentropus* + Dualulidae) + (basal Diptera + Siphonaptera); brown = *Parapolycentropus* + Dualulidae; black = basal Diptera and Siphonaptera

separated. Prothorax and metathorax small, mesothorax comparatively enlarged. Legs slender; two claws at the end of pretarsus. Forewing long, ovoidal, slightly rounded apex. Sc relatively short, extending to C near Rs bifurcation, with an anterior branch slightly distal to or at same level of R bifurcation. $R_1$ single and extending much beyond $Rs_{1+2}$ forking; Rs with four branches, $Rs_{1+2}$ forking considerably distal to $Rs_{3+4}$; Rs forking proximal to M. M with four branches, $M_{1+2}$ bifurcation considerably distal to $M_{3+4}$; Rs originating from $R_1$ distinctly distal to M from CuA. CuA and CuP single; stem of M curved with an almost right angle; anal area relatively narrow, two or three anal veins present; two crossveins between CuA and CuP; one a1-a2 present. Thick, short setae on the membrane from R forking to tip of wing; several long setae on entire margin. Hind wing degraded to a minute, tubular-shaped lobe. Female abdomen with 11 segments, but male only nine visible segments. Female cercus with two segments; male ameristic. Male claspers very robust, with bent dististylus.

**Included genus**. Type genus only.

### Genus *Dualula* Lin, Shih, Labandeira and Ren, gen. nov.

**Type species**. *Dualula kachinensis* Lin, Shih, Labandeira and Ren, sp. nov. (Figs. 1–3, Supplementary Figs. 2-5).

**Etymology**. The generic name refers to a combination of *duo-ae* (Latin, meaning 'two' or 'dual') and *–alula-ae* (Latin, a diminutive variant of wing, *ala-ae*, meaning 'a tiny wing' or 'a small appendage'). This designation refers to the highly miniaturized hind wings of this genus. The gender is female.

**Diagnosis**. As for the family by monotypy.

### *Dualula kachinensis* Lin, Shih, Labandeira and Ren, sp. nov.

**Etymology**. The specific epithet is derived from the northern state of Kachin in Myanmar, where the first discovered species of *Dualula* was found, Latinized to *Kachin -ensis*.

**Diagnosis**. As for the genus by monotypy.

**Holotype**. See Figs. 2 and 3; specimen CNU-MEC-MA-2014001. A female with partially preserved body and forewings; complete proboscis and hind wings: Left forewing length at least 9.84 mm, width 2.63 mm; right forewing length 9.68 mm, and width 2.55 mm. Body length 8.12 mm (excluding proboscis and antennae). Proboscis length 3.23 mm; right antenna length at least 2.23 mm.

**Paratypes**. See Supplementary Figs. 2–5, specimens CNU-MEC-MA-2017016 and CNU-MEC-MA-2017017. Female with almost completely preserved body and wings; CNU-MEC-MA-2017016: Right forewing length 9.27 mm, width 2.30 mm; left forewing length 8.78 mm, and width 2.25 mm. Body length 8.4 mm (excluding proboscis and antennae). Proboscis length (as preserved) 2.72 mm; right antenna length 2.13 mm.

Male with completely preserved body and wings, CNU-MEC-MA-2017017: Right forewing length 7.35 mm, width 1.87; left forewing length 6.85 mm, width 1.91 mm. Body

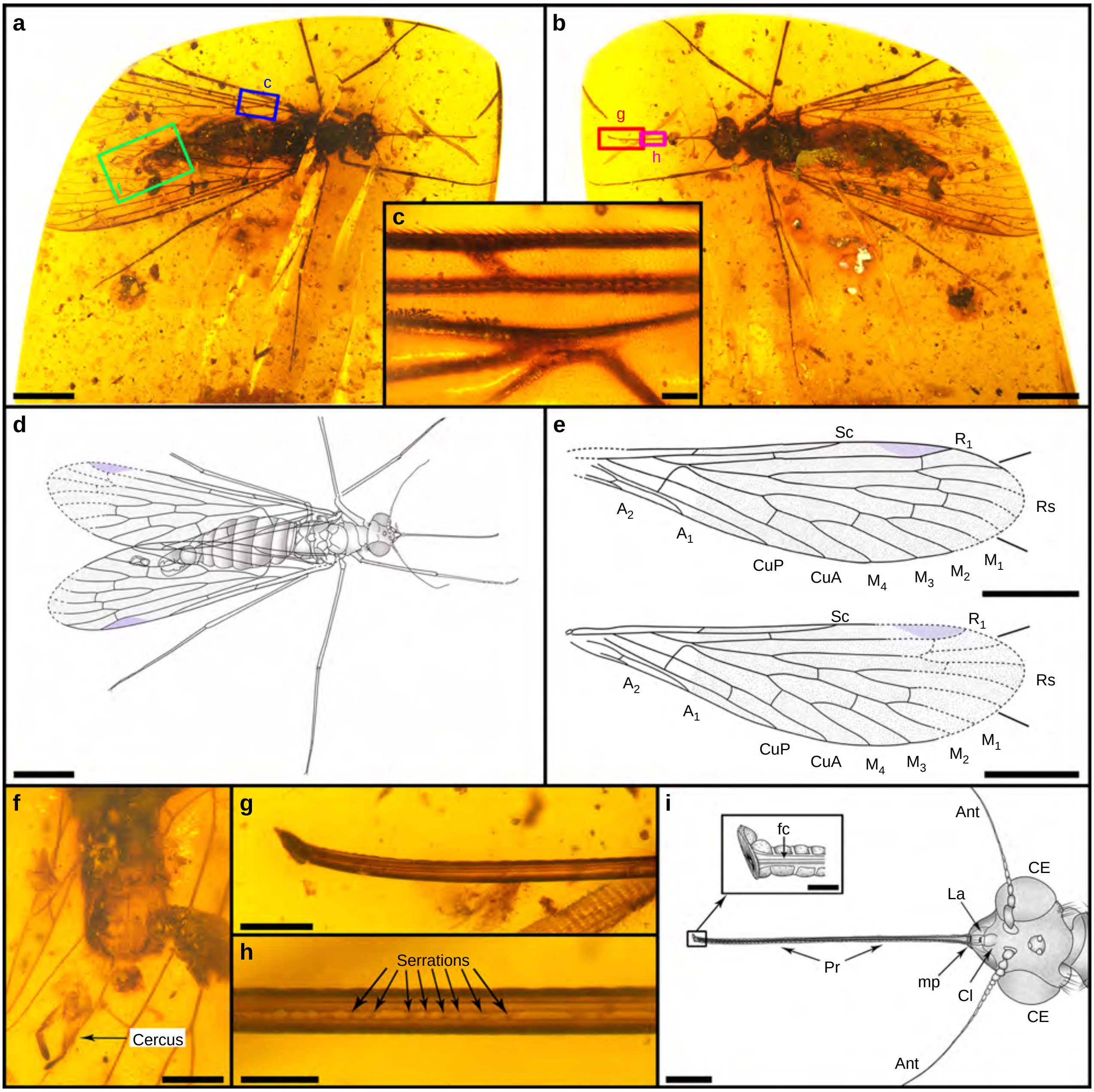

**Fig. 2** Photos and line drawings of holotype with details of the proboscis and genitalia. *Dualula kachinensis* gen. et sp. nov., CNU-MEC-MA-2014001, female. **a** Holotype in dorsal view. **b** Holotype in ventral view. **c** Details of setae on the margin and membrane of the left forewing, enlarged from blue template in **a**. **d** Overlay drawing of holotype in dorsal view. **e** Line drawing of forewings. Above is the right forewing and below is the left forewing. **f** Female genitalia in ventral view, enlarged from green template in **a**. **g** Proboscis terminus, with details in ventral view, enlarged from the larger magenta template in **b**. **h** Proboscis midsection in ventral view, enlarged from the smaller magenta template in **b**. **i** Overlay drawings of the head and proboscis tip details, based on **a**. Ant antenna, CE compound eye, Cl clypeus, fc food canal, La labrum, mp maxillary palp, and Pr proboscis. Scale bars represent 2 mm in **a**, **b**, **d** and **e**; 0.5 mm in **f** and **i**; 0.2 mm in **g**; 0.1 mm in **c** and **h**; and 0.05 mm in proboscis tip from **i**

length 7.62 mm (excluding proboscis and antennae). Proboscis length 1.82 mm; left antenna length 2.08 mm.

**Horizon and Locality**. Hukawng Village, Kachin State, northern Myanmar; Upper Cretaceous (earliest Cenomanian), 98.79 ± 0.62 Ma[18].

**Description**. See Figs. 2–4; Supplementary Note 1; Supplementary Figs. 2–5; Supplementary Data 4.

Further systematic paleontological details of the newly described taxon, *Dualula kachinensis* gen. et sp. nov., related *Parapolycentropus*, and associated issues are provided in Supplementary Note 4. Linked figures showing morphological features of the mouthparts, head, thorax, wings, legs, abdomen, and genitalia are given in Figs. 1–3 and Supplementary Figs. 2–5. The preservational status and geological provenance of 13 Pseudopolycentropodidae species are given in Supplementary Table 1.

**The long-proboscid condition in Aneuretopsychina**. Currently, there are four commonly occurring groups of long-proboscid, siphonate insects[24]. The first group consists of moths and butterflies (Glossata), a major defining feature is the siphonate proboscis[25]. The second group consists of about eight, major

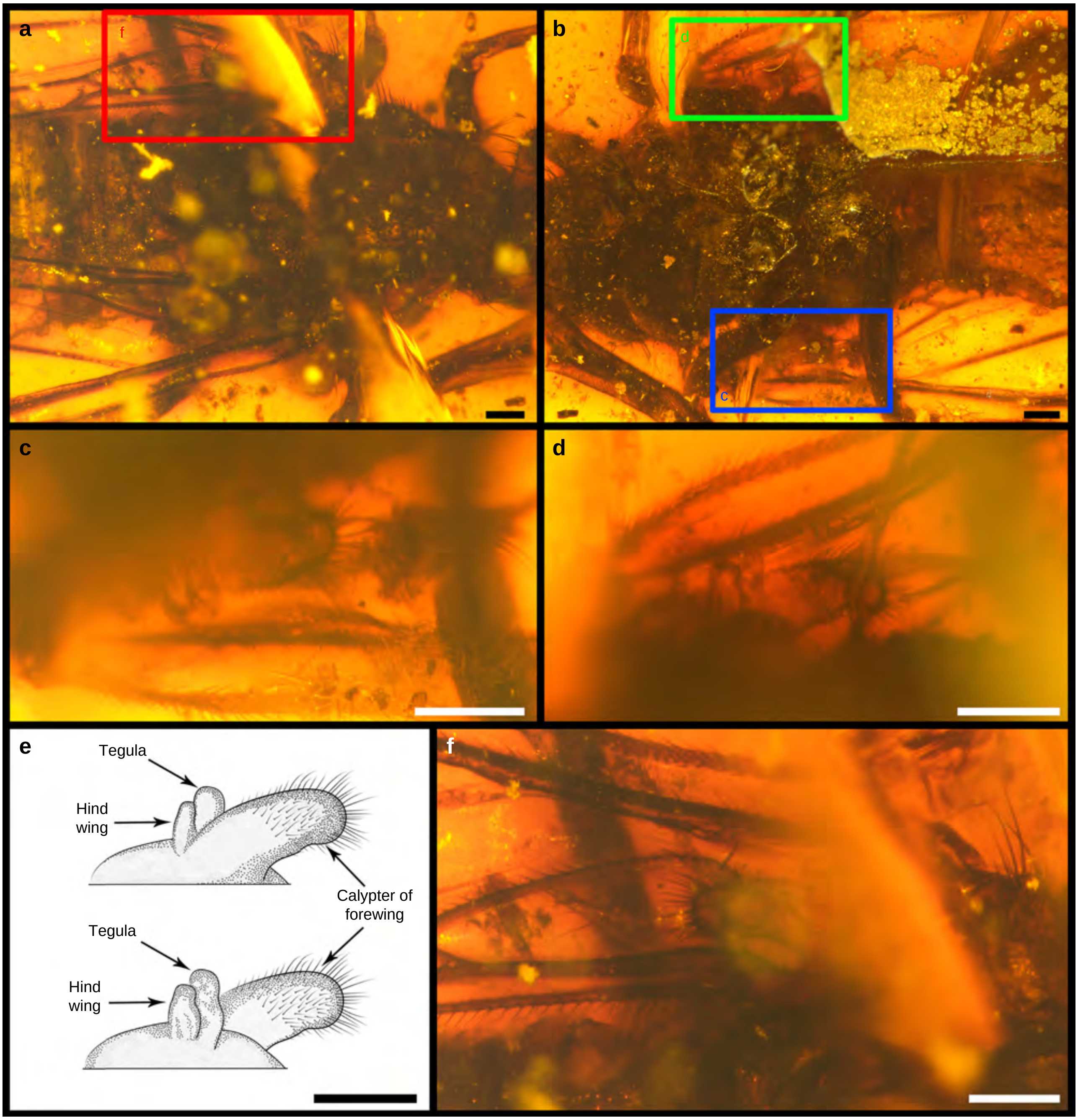

**Fig. 3** Hind wings of holotype *Dualula kachinensis* gen. et sp. nov. of CNU-MEC-MA-2014001. **a** Thorax in dorsal view. **b** Thorax in ventral view. **c** Right hind wing in ventral view, enlarged from blue template in **b**. **d** Left hind wing in ventral view, enlarged from green template in **b**. **e** Line drawings of left hind wing, above in dorsal view and below in ventral view. **f** Left hind calypter in dorsal view, enlarged from the red template in **a**. Scale bars represent 0.2 mm in **a**–**f**

family-level fly lineages in the Brachycera, most of which convergently evolved similar long-proboscid mouthparts, although differences exist in overall form, aspect ratio and surface ornamentation[26,27]. Two other groups of modern, long-proboscid insects are Coleoptera (beetles) and Trichoptera (caddisflies) that occasionally evolved the long-proboscid condition in nonspeciose lineages[28,29]. Modern bearers of long-proboscid mouthparts are similar to the spectrum of groups that possessed long-proboscid mouthparts from the Middle Jurassic to mid-Cretaceous (170 to 95 Ma)[1]. The fourth, long-proboscid, mid-Mesozoic groups consisted of major extinct lineages within Mecoptera[5,6,17] and Neuroptera[8,9,21]. By contrast, mid-Mesozoic long-proboscid lineages of Diptera[12,13] and Lepidoptera[10,11] overwhelmingly are extant. It is notable that of the four mid-Mesozoic groups, Mecoptera and Neuroptera no longer have long-proboscid forms, and often are biogeographical relicts[9] (Supplementary Note 2).

Within Mecoptera, the historically defined Aneuretopsychina[14] contains four, major, long-proboscid lineages: Nedubroviidae[2], Mesopsychidae[30], Aneuretopsychidae[11] and Pseudopolycentropodidae[20]. The Nedubroviidae[2] are an obscure late Permian to Middle Triassic group for which few details of the head and mouthparts are known, other than a prominent triangular labrum and an incomplete 0.32 mm long proboscis with a food canal that

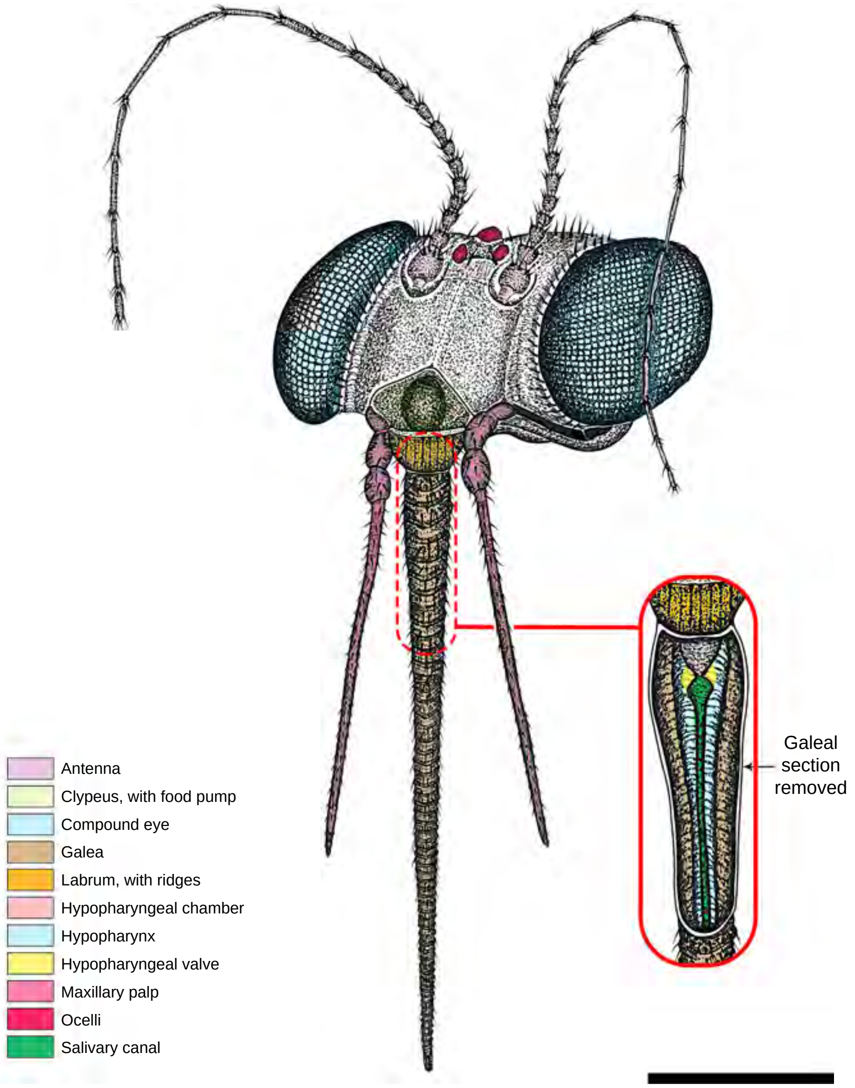

**Fig. 4** Reconstruction of head and mouthparts of male *Dualula kachinensis* gen. et sp. nov. The drawing is based mostly on specimen CNU-MEC-MA-2017017, supplemented by CNU-MEC-MA-2014001 and CNU-MEC-MA-2017016. A section representing the upper proximal third of the galeae has been removed to reveal features below of the pharyngeal pump. This subfigure was created from microscope photographs of the amber specimen by Conrad Labandeira as a hand drawing modified in Adobe Photoshop CC by Xiaodan Lin. Scale bar represents 0.5 mm

is missing a terminus. Better preserved Mesopsychidae are a late Permian to Early Cretaceous lineage bearing a long, forwardly directed (prognathous), siphonate proboscis constructed of maxillary galeae interlocked by a tongue-and-groove suture and housing a central food tube[6,30]. The proboscis terminus houses two, laterally placed ovoidal pseudolabellae and an up to a 11.2 mm long proboscis shaft that has an external surface covered, sometimes sparsely, by randomly positioned, thick setae, but lacking other ornamentation such as transverse ridges. At the proboscis base are laterally positioned, adpressed, three-articled maxillary palps and a domed clypeus suggesting a cibarial pump. Direct evidence for a hypopharynx (pharyngeal pump and salivary duct) is lacking, although functional considerations and the presence of a third proboscis element between separated galeae in one specimen indicates its presence[6].

The known proboscis of Aneuretopsychidae is 8.5 mm long and directed rearward (opisthognathous), a feature differing from all other Aneuretopsychina[6,14,19]. The proboscis has an outer surface of transverse, annular ridges bearing perpendicularly placed robust setae, and a terminus with a fleshy, U-shaped pseudolabellum wrapped around an ellipsoidal mouth[6,19]. A cibarial pump is present in Aneuretopsychidae, in addition to a second, smaller, labral pump probably homologous to the pharyngeal pump in the Dualulidae. In contrast to Mesopsychidae and Aneuretopsychidae, the much smaller, up to 2.2 mm long, forwardly projecting proboscis of Pseudopolycentropodidae bore on its outer surface obscure, regularly spaced, sclerotized annular rings with microtrichia and lacked pseudolabellae on the terminus[16,17,20]. There is evidence for a hypopharynx in compression Pseudopolycentropodidae, although its specific structure remains unknown. Other mouthpart elements, such as the labrum and labium, were used principally as proboscis braces. The maxillary palp consisted of three articles, the terminal one longer than the other two (Supplementary Note 2; Supplementary Fig. 7; Supplementary Data 4, 5). *Parapolycentropus* possessed a distinct cibarial pump under the clypeal region and a proboscis similar to other compression Pseudopolycentropodidae[16,17]. The proboscis bore setae and sclerotized bands encircling the outer surface. A distinct hypopharynx housed a bulbous pharyngeal pump connected distally to a salivary duct, although there is no evidence for a valve. The salivary duct had diminutive ventral serrations and terminated at the proboscis tip. The maxillary

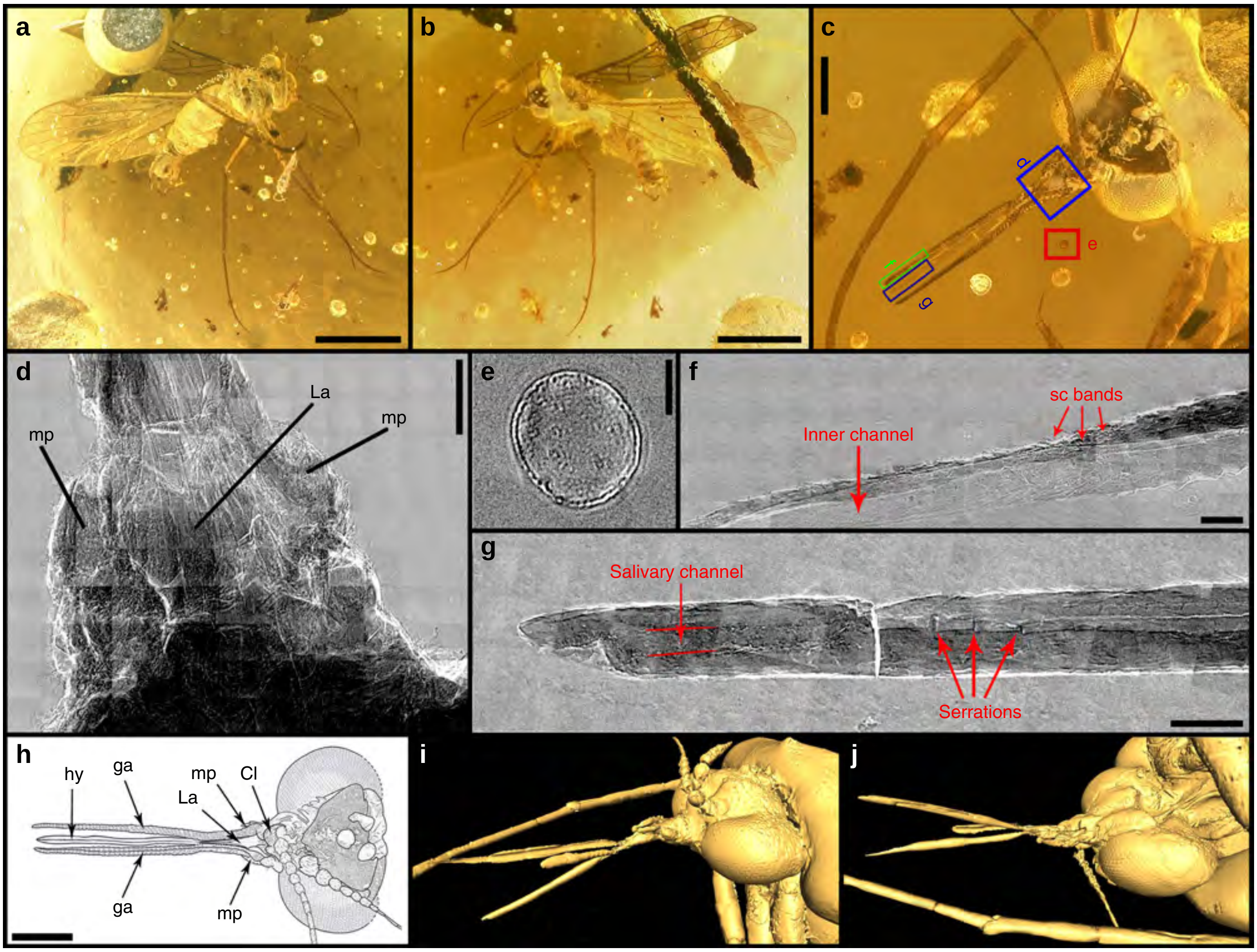

**Fig. 5** Nano-CT and Micro-CT images of *Parapolycentropus paraburmiticus*[17] head and associated pollen grain. Specimen CNU-MEC-MA-2015054; new material, male. **a** Insect in right lateral view. **b** Insect in left lateral view. **c** Head and mouthparts in dorsal view. **d** Nano-CT images of the proboscis base in ventral view, from the blue template in **c**. **e** Nano-CT images of a likely pollen grain near the right galea from the red template in **c**. **f** Nano-CT images of the left galeal tip from the green template in **c**. **g** Nano-CT images of the hypopharynx tip and associated external ornament in dorsal view, from the black template in **c**. **h** Line drawing of the head and proboscis in **c**. **i** 3-D reconstruction of head in lateral view from a micro-CT scan. **j** The same 3-D reconstruction of head in **i**, except in ventral view. Images **i** and **j** are from Micro-CT scanning, reconstructed in Amira software. Cl clypeus, ga galea, hy hypopharynx, La labrum, mp maxillary palp, sc bands sclerotized proboscis bands. Scale bars represent 1 mm in **a** and **b**; 0.2 mm in **c** and **h**; 30 µm in **d**; 10 µm in **e**; 15 µm in **f**; and 20 µm in **g**. Scale bars are absent in **i** and **j**

palps were similar to other Aneuretopsychina, short and consisting of three articles.

**Novel mouthparts of *Dualula*.** The feeding mechanism of *Dualula* consists of a pharyngeal pump linked by a valve to a salivary duct that provides controlled, outgoing salivary secretions. The pharyngeal pump–valve–salivary duct system is lodged within a food tube of much wider diameter. The food tube accessed incoming fluid food and was powered by a cibarial pump under the clypeus (Figs. 2 and 4; Supplementary Figs. 3a–e; 5a, b). This condition indicates a dual pump system that worked with fluids in the food tube and salivary duct secretions flowing in opposite directions. Recently, such a dual pump mechanism was considered possibly present in some long-proboscid Aneuropsychina[1,6], including Pseudopolycentropodidae based on compression-impression fossils from Northeastern China[6,20]. A dual pump system is better documented for *Parapolycentropus* (Fig. 5; Supplementary Figs. 8, 9). Similarly, the terminus of the *Dualula* proboscis was blunt, similar to a truncated straw end and lacked pseudolabellae or other terminal structures for sponging surface fluids by capillary action[6,10,19] (Fig. 2g, i; Supplementary Fig. 5b). For a small, mosquito-sized insect such as *Dualula*, initial mobilization by enzyme-laden secretions of viscous surface fluids hidden in channels, funnels or other tubular structures of ovulate organs would have been an effective mode of ingesting pollination drops (Supplementary Note 3).

Four observations provide evidence for the function of the *Dualula* and closely related *Parapolycentropus* proboscis. The observations concern: (i) proboscis aspect ratios and diameters, (ii) proboscis cross sections, (iii) multiple longitudinal views of proboscis element surfaces, and (iv) expelled fluids from the food tube and salivary duct. First, *Dualula* possessed a proboscis considerably smaller and more slender than other long-proboscid scorpionflies of the mid Mesozoic. The average *Dualula* proboscis diameter was 0.12 mm, with a range of 0.10–0.16 mm. As a measure of slenderness, the average aspect ratio (proboscis length divided by its width) was 24.42 for a male and two female specimens. When compared to other long-proboscid, mid-Mesozoic scorpionflies such as Mesopsychidae with a much larger average body length (excluding antennae and proboscis) of 22.6 mm (Supplementary Data 4), the proboscis aspect ratio is

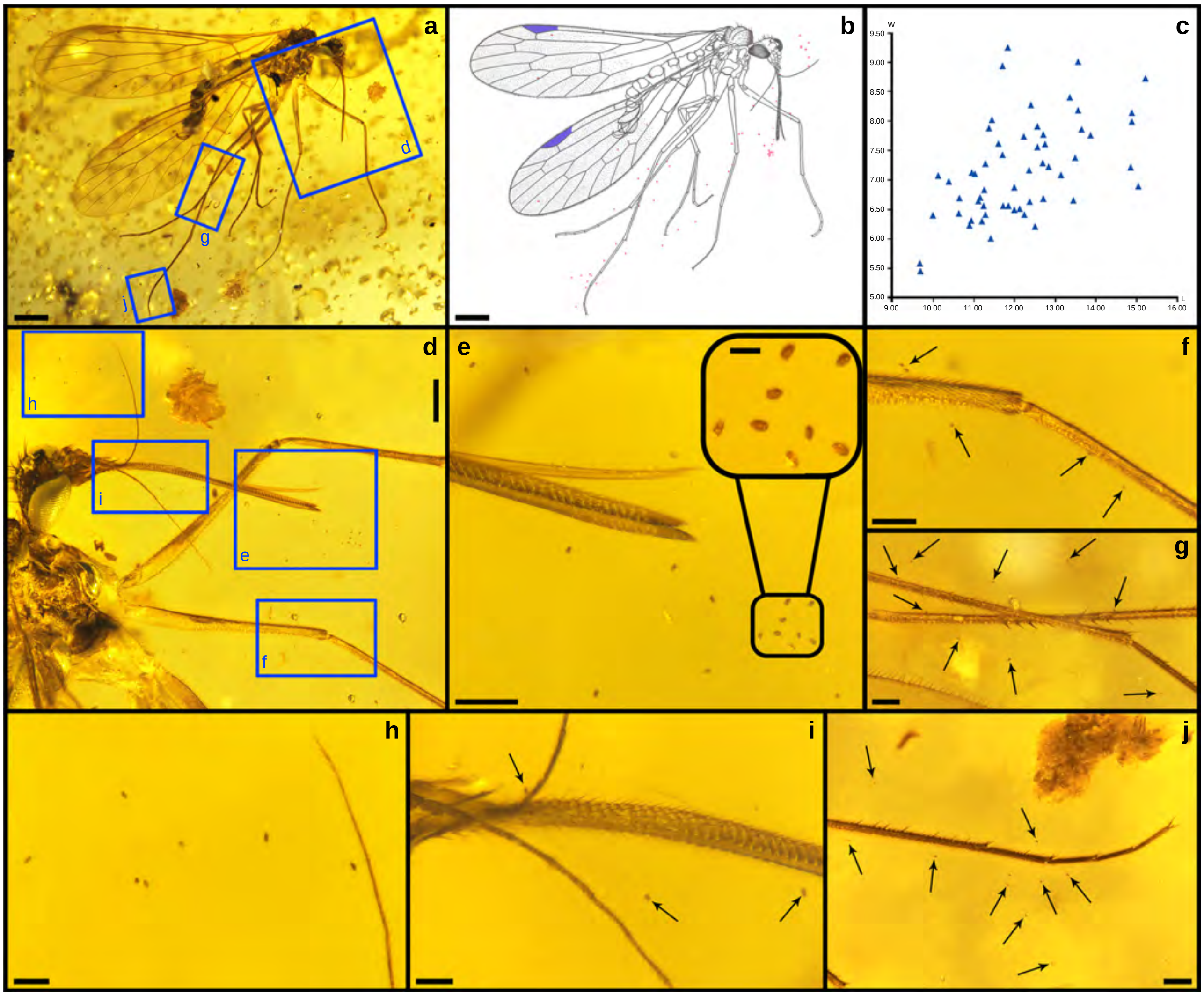

**Fig. 6** A male *Parapolycentropus paraburmiticus*[17] associated with Cycadopites sp. pollen grains. Concentrations of pollen surround the mouthparts, antennae, legs and wings (CNU-MEC-MA-2017012, new material). **a** The insect specimen. **b** Line drawing of the entire insect in **a** surrounded by *Cycadopites* pollen grains, shown as tiny red dots. **c** Plot of *Cycadopites* dimensions, shown as length (L) along the horizontal axis and corresponding width (W) along the vertical axis. The pollen-grain measurement data is from **a** and **b**, available in Supplementary Data 3. **d** Head, prothorax and proximal forelegs, enlarged from template in **a**. **e** Proboscis tip with galeae and hypopharynx surrounded by pollen grains, enlarged from **d**. Enlargement of several pollen grains near the proboscis tip at right. **f** Enlargement of right foreleg in **d**, with arrows pointing to nearby *Cycadopites* grains and clumps. **g** Enlargement of the right middle and hind legs in **a**, showing adjacent pollen indicated by arrows. **h** Pollen grains near the antennal tip outlined in **a**. **i** Proximal aspect of the proboscis and associated mouthparts with three pollen grains indicated by arrows. **j** Tarsus of right hind leg, indicted in **a**, with adjacent pollen indicated by arrows. Scale bars represent 0.5 mm in **a** and **b**; 0.1 mm in **e**–**g** and **j**; 0.2 mm in **d**; 50 μm in **h** and **i**; and 20 μm in-group of pollen grains from **e**

very similar at 22.46, but diameters are from three to four times that of *Dualula*. Comparisons of other lineages of Aneuretopsychina to *Dualula* indicate average proboscis aspect ratios are substantially lower and proboscis average diameters are considerably different. The average proboscis aspect ratio is 16.31 for Aneuretopsychidae; the average diameter is 0.35 mm (range 0.29–0.41 mm), about three times that of *Dualula*. For Pseudopolycentropodidae the aspect ratio is 13.02; the average diameter is 0.16 mm (range 0.08–0.25 mm), about 1.33 times wider than *Dualula*. For *Parapolycentropus* the aspect ratio is 14.03; the average diameter is 0.07 mm (range 0.04–0.11 mm), significantly narrower than *Dualula* (Supplementary Data 4 and 5). These variable proboscis aspect ratios and diameters strongly indicate accommodation to a variety of receiving diameters of tubular structures from a spectrum of contemporaneous gymnosperm ovulate organs and possibly small angiosperm flowers[1,6] (Supplementary Fig. 11).

Second, a female *Dualula* proboscis is transversely cut where it intersects the amber surface (Supplementary Fig. 3e). This cross-section displays an expansive space between the inner surface of the galeal food tube and the outer surface of the encompassed salivary duct. The small salivary duct displays a very narrow, inner tube diameter. Third, longitudinal views of the outer surface of the galeal food tube and the salivary duct clearly is observed in several *Dualula* specimens (Fig. 2g, h; Supplementary Fig. 5a, b). The lengths of these salivary ducts are ca. 6–9 mm long and their inner diameters are about one third of their outer widths (Supplementary Fig. 3e).

Fourth, fluids are shown expelled from food tubes and salivary ducts soon after resin entombment, resulting in bubbles. Three

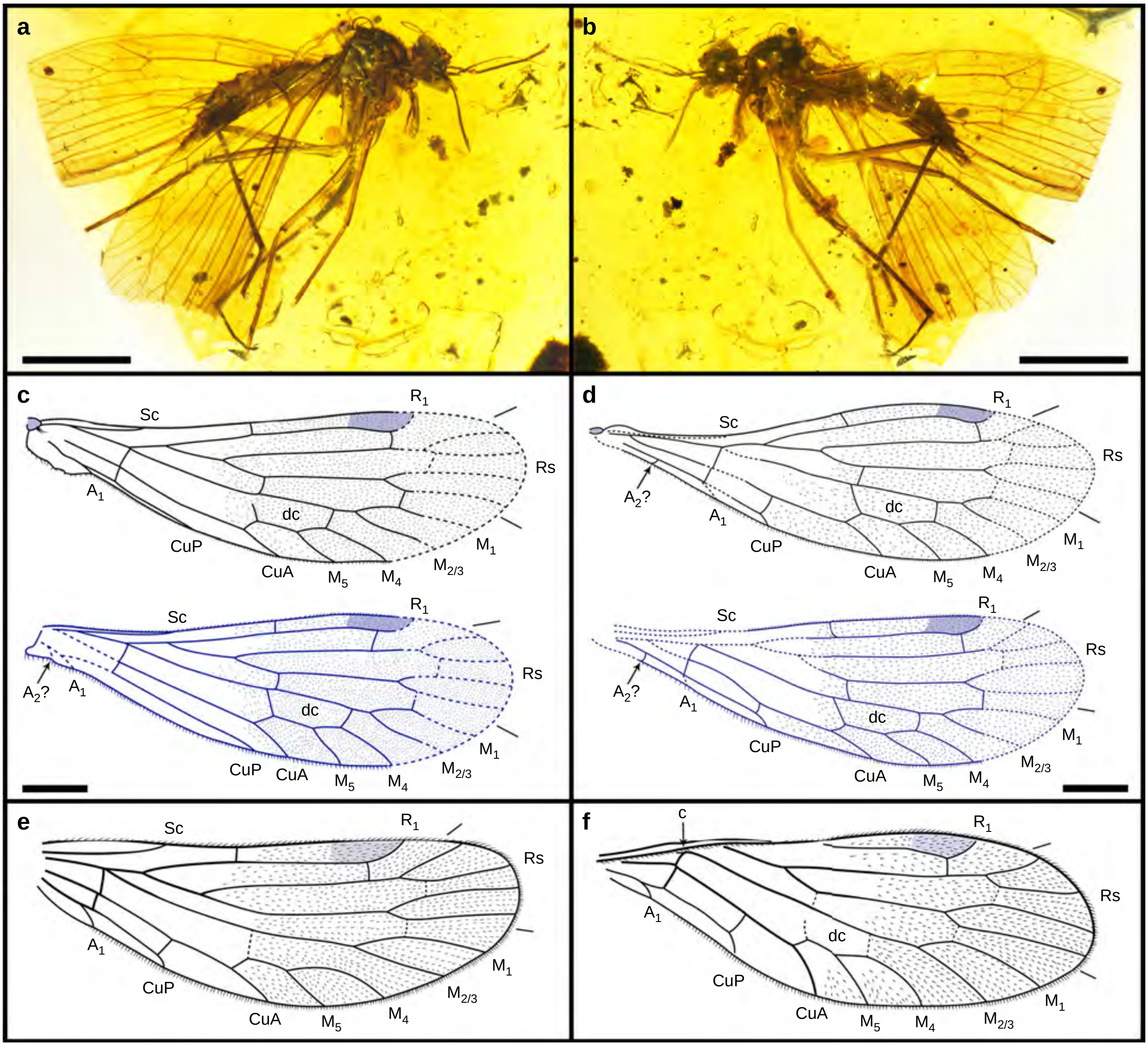

**Fig. 7** Photos and line drawings of a female four-winged *Parapolycentropus*. CNU-MEC-MA-2017006, new material with the redrawn images of forewings of two species of *Parapolycentropus*. **a** Specimen in right lateral view. **b** Same specimen at **a** in left lateral view. **c** Line drawings of right wings. **d** Line drawings of left wings. **e** Reconstructed forewing of *P. burmiticus*. **f** Reconstructed forewing of *P. paraburmiticus*. In **c** and **d**, forewings are black and hind wings are blue. Wings **e** and **f**, based on a published reconstruction in figure 8b and 8d of Grimaldi et al.[17]. Scale bars represent 1 mm in **a** and **b**, and 0.5 mm in **c**, **d** Subfigures **e** and **f** lack scale bars

examples show distinct fluid emissions in *Parapolycentropus* from the larger food tube (Supplementary Fig. 8p–r). In one example (Supplementary Fig. 8p), there is a bubble of food-tube fluid (top center) and a much smaller bubble of salivary-duct fluid (left center) from the same proboscis. These four types of evidence suggest not only presence of a double pump system, but also separate inflowing fluids into the food tube and outgoing secretions from a much narrower salivary duct (Supplementary Figs. 8i–m; 9d, e, g, h; Supplementary Movie 1).

**Feeding biology on host plants**. Two types of evidence are available for inferring the feeding biology of *Parapolycentropus* and *Dualula*. The first type is indirect evidence of structural features is consistent with proboscis probing and uptake of fluids from gymnosperm and angiosperm reproductive organs. Such evidence includes features of long-proboscis surfaces and suspect gymnosperm and angiosperm reproductive organs that allow reception and accommodation of a proboscis. The second type is direct evidence demonstrating close association of pollen with body surfaces of insects[1,4,10,13,31,32]. Particularly important is identification of pollen to a source plant in the same deposit whose biology is consistent with insect pollination[4,13,32].

We examined 77 well-preserved specimens of *Dualula* and *Parapolycentropus*. Two specimens of *P. paraburmiticus* were associated with pollen grains adjacent their bodies. The first specimen had associated pollen grains of a gymnosperm (Fig. 6), and the other an unknown inaperturate grain (Fig. 5c, e). Pollen from the first specimen was distinctive and consisted of 54 smooth, monosulcate grains, olive-shaped in polar view, boat-shaped in longitudinal equatorial view and kidney-shaped in short equatorial view (Fig. 6e). The pollen is distinctly monosulcate, with the sulcus membrane thickening toward the margin, and unusually small, characterized by an average length of 12.15

μm (range 9.69–15.21 μm), an average width of 7.17 μm (range 5.46–9.24 μm) and an average length-to-width ratio of 1.70 (range 1.28–2.18 μm) (Supplementary Data 3). Based on these measurement and structural features, the pollen grains are attributed to *Cycadopites*[33], a gymnosperm form genus[34] (Supplementary Note 4). *Cycadopites* pollen is affiliated with Cycadales, Peltaspermales, Ginkgoales, Czekanowskiales, Pentoxylales and Bennettitales[34–37]. Some in situ *Cycadopites* from Bennettitales are quite small (down to 16 micrometers) and may be the source of the minute grains associated with *Parapolycentropus*[34]; however, other clades, especially Cycadales, cannot be ruled out. This occurrence provides direct evidence for a *P. paraburmiticus* feeding habit on pollination drops, indicating a pollinator relationship[38].

A single, possible pollen grain was found adjacent the proboscis of a *P. paraburmiticus* specimen (Fig. 5c, e). The surface details of this grain were distinct, based on a clear Nano-CT image (Fig. 5e). It is inaperturate, scabrate, nearly spherical, and 25.49 μm long by 22.15 μm wide. The affiliation of this grain is unclear. In addition, an examination of a large collection of Myanmar amber yielded 12 pieces with five well-preserved angiosperm flower morphotypes (Supplementary Fig. 11; Supplementary Data 6). The pieces contained one to a few flowers, and one included a branchlet of several clustered flowers (Supplementary Fig. 11k). Most of the flowers belong to *Tropidogyne*[39] (Supplementary Fig. 11a, b, e–i, l–n, u), a possible member of the Cunoniacae (wild alder family), an extant family of early-derived, arborescent, dicotyledonous angiosperms of Oxalidales[40] with a Gondwanan distribution. *Tropidogyne* consists of two species – *Tropidogyne pikei*[39] and *T. pentaptera*[41]. Both flowers are cup-shaped, apetalous, bear five sepals, and house a ribbed, inferior ovary with a nectary disc and dark glands at the termini of floral appendages, features associated with insect pollination[31,42–44]. Members of Cunoniaceae produce tricolporate pollen, so clearly are not the producers of the grain associated with *P. paraburmiticus*. Two other cup-shaped flowers of unknown affinity are present (Supplementary Fig. 11c, d, j, k, v–z), designated Morphotypes A and B, that have features consistent with insect pollination[42–44] (Supplementary Note 4).

The longest, measured proboscis lengths for *Parapolycentropus paraburmiticus* is 1.53 mm and *P. burmiticus* 1.50 mm, which easily was accommodated, for example, by flower Morphotype A, a cup-shaped flower with an average sepal length of 2.44 mm but a likely corolla depth of about 1.55 mm (Supplementary Data 6). This floral distance from the top to the bottom of the corolla is in accord with the proboscis length of *P. paraburmiticus*, allowing for a reasonable 0.9 mm elevation of the gynoecium at the corolla base. Corolla depths of more bowl-shaped flowers of *T. pikei* and *T. pentaptera* were 1.89 mm and 1.97 mm, respectively, which would have accommodated proboscis lengths of both *Parapolycentropus* species. However, both *Tropidogyne* species would not have accommodated the much longer proboscis of *Dualula* that extended to 3.23 mm in one complete specimen (Supplementary Data 4). Modern, *Tropidogyne*-type flowers are consistent with an early-grade, basal angiosperm pollination mode typified by "small, bowl-shaped, white to yellowish, actinomorphic flowers, exposed sexual organs, perianth of separate sepals and petals [and] often clustered in inflorescences"[31]. Based on the indirect evidence of morphological features of *Tropidogyne* flowers and the direct evidence of gymnosperm pollen adjacent *Parapolycentropus* scorpionflies, this suggests that the pollinators of these two associations – one a gymnosperm host and the other several structurally similar angiosperm hosts – belonged to two taxonomically different insect pollinator guilds[1,4,31,43,44]. This suggests the plant host of *Dualula* was a gymnosperm ovulate organ[1,38] and the pollinator of *Tropidogyne* and similar flowers may have been a small *Parapolycentropus*, but more likely syrphid and muscoid flies with sponging labellae[31,44]. These observations support: (i) pollinator activity between *Parapolycentropus* and gymnosperms; (ii) between *Tropidogyne* and related angiosperms and *Parapolycentropus*, syrphid flies and especially muscoid flies; and (iii) between *Dualula* and an unknown gymnosperm host[1,4,6,31,38,43,44].

**Hind wing evolutionary developmental biology**. Like proboscis uniqueness, vestigial hind wings also are a relevant feature that characterizes *Dualula* and *Parapolycentropus*. Extant and extinct lineages of scorpionflies normally bear two pairs of approximately equal sized and structurally similar membranous wings on the mesothorax and metathorax[5,22]. However, *Parapolycentropus* and *Dualula* from Myanmar amber (Supplementary Table 1; Supplementary Data 4 and 5) possess highly modified hind wings[16,17]. Furthermore, one genus of Liassophilidae (*Laurentiptera*)[45,46] and 11 described species of compression Pseudopolycentropodidae also bear hind wings reduced in size and venation. These related taxa often bear hind wings considerably smaller than their forewings and occasionally resemble halteres (Supplementary Fig. 12a, d); the hind wings of *Dualula* also have been reduced to small, haltere-like structures (Fig. 3c–e).

Of taxa with highly modified wings, one amber specimen possessed four wings of a nominal two-winged *Parapolycentropus* (Fig. 7a–d). The fore-and hind wings of this four-winged variant were identical in features to typical, four-winged scorpionflies past and present. The size, shape and venation of this specimen's wings are nearly identical between fore- and hind wings, minus minor differences of the anal area between right and left wings (Fig. 7c, d). No significant morphological or venational differences occurred between the forewings of the four-winged specimen and other specimens of this genus (Fig. 7e, f). The four-winged specimen demonstrates that hind-wing reduction is possible within a low-ranked, major lineage of Mecoptera (Fig. 7), an observation pertaining to Pseudopolycentropodidae, Liassophilidae, *Parapolycentropus* and *Dualula*. Given the protracted history of Mecoptera, the establishment of hind-wing reduction is a recurring evolutionary developmental pattern, explained by regulation of the *Ultrabithorax* Hox gene[47,48], transcription factors and regulatory cascades (Supplementary Note 5). The transition of Mecoptera hind wings from broad, membranous structures to small, narrow, haltere-like structures is a key structural acquisition that likely accelerated diversification of the group during the early Mesozoic, and continued as a ground-plan feature in earliest Diptera[49] (Supplementary Note 6).

**Male genitalia structure**. Male genitalia of one particular extant scorpionfly, Panorpidae (common scorpionflies), features a prominent structure arched over its abdomen resembling a large scorpion sting. Although scorpioid male genitalia is atypical for extinct and extant Mecoptera[50], mid-Mesozoic taxa of Aneuretopsychina, particularly Mesopsychidae[30,51], Pseudopolycentropodidae[52], *Parapolycentropus*[16] and *Dualula*, also exhibit unique, male genitalic homologies not found in other Mecoptera or Diptera. Distinctively homologous features of Pseudopolycentropodidae (Supplementary Fig. 12) and Mesopsychidae (Supplementary Figs. 13,14) from the Middle Jurassic include an upturned gonostylus, a very robust gonocoxa and dististylus (claspers), a terminal concavity on the dististylus, and undifferentiated cerci. These features were retained in most *Parapolycentropus* (Figs. 6a, b and 7a, b; Supplementary Figs. 9a, b; 15c,d) and *Dualula* (Supplementary Fig. 5c–e) from mid-Cretaceous Myanmar amber, showing a 65 million-year-long evolutionary continuity of genitalic structure linking Eurasian Mesopsychidae, Pseudopolycentropodidae, *Parapolycentropus* and *Dualula*.

**Reproductive biology**. Fossil discoveries rarely provide insight into insect group behavior of the deep past. An exception is occasional pieces of amber that entomb a population of numerous, conspecific individuals engaged in congregation, such as mating or dispersal. Three Myanmar amber pieces preserve such swarming behavior and indicate a coordinated congregation of conspecific individuals that typically involve flies such as nonbiting midges[53]. One mode of swarming behavior especially ubiquitous in many nematocerous fly lineages is lekking, a midair assembly of flies typically within a few meters of the ground surface, involved in a communal mating event. This phenomenon rarely has been documented in scorpionflies, but examples are known from modern Bittacidae[54] and fossil Nannochoristidae[55]. Because most Pseudopolycentropodidae, *Parapolycentropus* and *Dualula* species are mosquito sized and Myanmar amber pieces occasionally approach or exceed 5 cm in length, entombment of a swarm of lekking individuals is a distinct possibility (Supplementary Note 7). Fortunately, three pieces of amber were identified with abundant *Parapolycentropus* specimens, consisting of 9, 18, and 4 individuals, with varying combinations of *P. burmiticus* and *P. paraburmiticus* and female to male ratios of 1:6, 1:2, and 2:1, respectively. This pattern indicates an absence of species specificity and varied sex ratios, suggesting that lekking behavior favored male mating swarms[56]. Mating may have occurred in more diffuse combinations of *Parapolycentropus* species and irregular sex ratios involving aerial copulation of a larger female and more smaller males (Supplementary Fig. 16a, b) in an end-to-end stance of connecting genitalia (Supplementary Fig. 16c,d). Such an unusual copulatory position also exists among extant Panorpodidae and Panorpidae[50,57] (Supplementary Note 7).

## Discussion

Our reanalysis of Mecoptera employed a comprehensive list of 51 characters on 37 taxa that establishes a robust hypothesis for phylogenetic placement of core Pseudopolycentropodidae, *Parapolycentropus*, *Dualula*, other long-proboscid Aneuretopsychina, other mid-Mesozoic and modern Mecoptera, and early Mesozoic Diptera and Siphonaptera. Several morphological differences separate *Dualula* (Dualulidae) from other families of Mecoptera that include unique proboscis construction, reduced hind wings and genitalic features. The results (Fig. 1) indicate that Dualulidae is the sister group of *Parapolycentropus* but also has close relationships with other long-proboscid Mecoptera of Aneuretopsychina, basal Diptera and Siphonaptera lineages. Based on the results of trees in Fig. 1, there are two major hypotheses for the origin of the long-proboscid condition in mid-Mesozoic Mecoptera. The first hypothesis is the long-proboscid condition originated twice. Long-proboscid mouthparts were acquired in the common ancestor of the Pseudopolycentropodidae + (Liassophilidae + {Permotanyderidae + [Aneuretopsychidae + ||Mesopsychidae + Nedubroviidae||]}) clade and separately in the *Parapolycentropus* + Dualulidae clade, indicated by the magenta and brown arrows, respectively, in Fig. 1. A twofold origin would require that long-proboscid mouthparts were retained in Liassophilidae (*Liassophila*)[58] and Permotanyderidae (*Choristotanyderus*)[59], but originated independently in the *Parapolycentropus* + Dualulidae clade. Accordingly, they became generalized and present as haustellate mouthparts in the ancestor to Diptera and Siphonaptera.

The second hypothesis is the long-proboscid condition evolved three times. The first origination was the core Pseudopolycentropodidae clade of *Pseudopolycentropodes* + (*Pseudopolycentropus* + *Sinopolycentropus*), indicated by the magenta arrow in Fig. 1. The second origination was the Aneuretopsychidae + (Mesopsychidae + Nedubroviidae) clade, indicated by the green arrow in Fig. 1. The third origination was the *Parapolycentropus* + Dualulidae clade, sister group to basal Diptera and Siphonaptera, indicated by the brown arrow in Fig. 1. This hypothesis presumes that Liassophilidae and Permotanyderidae retained the plesiomorphic condition of mandibulate mecopteran mouthparts. The threefold origin of long-proboscid mouthparts in Mecoptera is strongly favored here, because of distinct differences in proboscis construction among the three lineages[2,16,17,19,20,38,52].

Our phylogenetic result is similar to Ren et al.[6], but differs from other studies in four important aspects. First, basal Diptera are not the sister-group of Mecoptera, but rather originate within Mecoptera; Second, extant families of Mecoptera are not a monophyletic group, but exhibit paraphyly and polyphyly. Third, Aneuretopsychina are paraphyletic if Liassophilidae and Permotanyderidae lacked long proboscides. Fourth, *Parapolycentropus* is not a member of Pseudopolycentropodidae, but is a clade with *Dualula*, which in turn is the sister-group to basal Diptera + Siphonaptera. However, there are several limitations of our analysis. One issue is the lack adequate sampling, including all extinct and extant genera from families of Mecoptera and relevant Diptera. Second, the analysis is based on morphological data only. A third constraint was restriction of the data overwhelmingly to wing venation characters, which rendered insufficient resolution of Siphonaptera. To conclude, our research is a preliminary exploration of phylogenetic relationships among long-proboscid Mecoptera and relevant groups, and provides a framework for future studies. (These issues are discussed in Supplementary Note 1.)

The time of origin of the long-proboscid condition in Mecoptera, synonymous with the origin of the mid-Mesozoic clade Aneuretopsychina sensu lato[6,14], likely was late Permian. This timing is based on presence of Nedubroviidae[2], Mesopsychidae[3] and Liassophilidae[58] from late Permian to Middle Triassic deposits in Europe. However, Permotanyderidae, a likely member of the Aneuretopsychina, is known from the earlier late Permian of Australia[60]. Consequently, the earliest occurrences and likely place for the initial diversification of long-proboscid Aneuretopsychina was either northeastern Pangaea, on Baltica and Siberia, or northeastern Gondwana[61]. After this initial phase of modest speciation, a second phase of Aneuretopsychina diversification occurred during the Middle Jurassic to mid Cretaceous with multiplication of taxa in Mesopsychidae and Pseudopolycentropodidae, as well as speciation occurring in Aneuretopsychidae, *Parapolycentropus* and Dualulidae. This renewed diversification occurred in eastern Laurasia on the Tarim, Amuria, North China Block, South China Block and Annamia paleocontinents. These landmasses were docked earlier with eastern Laurasia or were separated by narrow oceanic gaps[61]. By mid Cretaceous, soon after Myanmar amber was deposited, the last lineages of the Aneuretopsychina became extinct, signaling the end of a 155 million-year legacy[1].

## Methods

**Localities and repositories**. This fossil study included 77 amber and seven compression fossil specimens. The amber specimens were collected from the Hukawng Valley of Kachin State, in northern Myanmar. The particular locality from which the specimens were collected was at the northern end of Noije Bum, which is a village located approximately at N26°150′, E96°340′, 18 km southwest of the town of Tanai. The amber is dated as earliest Upper Cretaceous (earliest Cenomanian), about 98.79 ± 0.62 Ma[18], equivalent to the early part of the Cenomanian Stage[62]. The compression fossils were collected from the latest Middle Jurassic Jiulongshan Formation at Daohugou Village, Shantou Township, Ningcheng County of Inner Mongolia, China. This locality is located at N41°18.979′, E119°14.318′, and has been radioisotopically dated at 164 Ma[63], corresponding to the later part of the Callovian Stage[62]. Most of the studied material is housed in the Key Lab of Insect Evolution and Environmental Changes, at the College of Life Sciences of Capital Normal University (CNU), in Beijing, China. Six specimens of CNU-MEC-MA-2015025, CNU-MEC-MA-2015027, CNU-MEC-MA-2015029, CNU-MEC-MA-2015030, CNU-MEC-MA-2015031 and CNU-MEC-MA-2015032 currently are on loan to CNU but will be returned to the Three Gorges Entomological Museum (EMTG), in Chongqing, China, where they finally will reside.

**Amber preparation**. All amber pieces were polished with emery paper sheets with varying grit sizes of 300, 600, 1000, 3000, 5000 and 7000 grit, in a time sequence of coarse to finer grit size. Care was taken to avoid contamination from sheets of different grit size. The amber finally was processed with Tamiya polishing compound@2004 TAMIYA for a smooth finish. For *Parapolycentropus paraburmiticus* (CNU-MEC-MA-2017012), the area of interest was polished close to the insect body surface for ease of viewing, while avoiding contact damage to body structures. However, the isolation of pollen grains was not feasible. Imaging of the *Cycadopites* sp. pollen grains by a Micro-CT scanner also proved unsuccessful, attributable to the poor absorptive capacity of X-rays from the lack of a density difference between the pollen and entombing amber.

**Specimen imaging**. Most of specimens were examined and photographed under a Nikon SMZ25 microscope attached to a Nikon DS-Ri2 digital camera system in the Fossil Insect Laboratory at CNU. Four specimens – CNU-MEC-MA-2016007, CNU-MEC-MA-2015030, CNU-MEC-MA-2015038 and CNU-PLA-MA-2016001 – were photographed under an Olympus DSX100 digital camera system. The equipment was the Scanning Electron Microscope (SEM) Laboratory of the National Museum of Natural History (NMNH), Smithsonian Institution, Washington, D.C. Photographs of other specimens, such as CNU-MEC-MA-2015054, were captured with a Z16 Leica®TM lens attached to a JVC KY-F75U digital camera system in the Department of Entomology Laboratory at NMNH[64–66]. This system was used to stack photos employing a series of software consisting of Cartograph 7.2.5®TM and Archimed®TM 6.1.4, and stacked with Combine ZP®TM. Incident lighting was used by techniques suggested in summaries of best procedures[64–66]. All photomicrographs with green background (Supplementary Fig. 5b) were taken by green epifluorescence as the light source, attached to a Zeiss Axio Zoom.V16 compound microscope, and with a fluorescence-image noise elimination system (Zeiss Apo Tome.2) in the College of Life Sciences public laboratory at CNU. Micro-CT scanning and three-dimensional reconstruction of specimens CNU-MEC-MA-2015054 and CNU-MEC-MA-2017008 were scanned with a Micro-CT (Nano Voxel 3000D, Sanying Precision Instruments Co., Ltd., Tianjin, China), located at the School of Mathematical Sciences at CNU. The voltage of the Micro-CT scanner was 50KV and the phase-contrast enhancement technique was used to reconstruct CT images with a higher contrast. The proboscis structures of the two above specimens were rendered with Amira@ 5.4.3 (Visage Imaging, San Diego, USA) and Avizo@ Fire 8.0 (Visualization Sciences Group; Massachusetts, USA). The Nano-CT images of specimen CNU-MEC-MA-2015054 – including the insect and pollen grain – were scanned with a Nano-CT (BL01B1) located in the National Synchrotron Radiation Research Center (NSRRC), in Hsinchu, Taiwan. Three SEM photos of specimens CNU-MEC-NN-2016001P, CNU-MEC-NN-2016008 and CNU-MEC-NN-2016015P were completed in the SEM Lab of the NMNH, under the PHILIPS XL 30 ESEM system. The figures were composited using Adobe Photoshop CC graphics software, and the line drawings were prepared by Adobe Illustrator CC and Adobe Photoshop CC graphics software.

**Measurements, abbreviations and terminology**. The lengths of the proboscides, wings and antennae were measured from the base to apex. The body lengths were measured from the apex of the head to the appendicular terminalia of the abdomen, excluding the antennae and proboscis. The widths of the proboscides were measured at their broadest dimension, excluding the labrum and maxillary palpus. The lengths of pollen grains were measured through the horizontal axis and widths were measured by the vertical axis approximately perpendicular to the horizontal axis.

The terminology of wing venation for Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae follows established nomenclature[16,17]. Corresponding abbreviations in the text and figures are the following. For wing venation: Sc subcosta, $R_1$ first branch of the radius, Rs radial sector, $M_1$ first branch of the media, $M_2$, second branch of the media, $M_3$ third branch of the media, $M_4$ fourth branch of the media, $M_5$ fifth branch of the media, $M_{2/3}$ second and third branches of the media, MA anterior media, MP posterior media, CuA anterior cubitus, CuP posterior cubitus, $A_1$/1A first branch of the anal vein, $A_2$/2A second branch of the anal vein, $A_3$ third branch of the anal vein, and dc central discal cell. For head and proboscis: Ant antennae, car cardo, CE compound eye, Cl clypeus, fc food canal, ga galea, hy hypopharynx, is inner surface of galea, La labrum, mp maxillary palp, oc ocellus, os outside surface of galea, Pr proboscis, sc sclerotized bands, sti stipes. For genitalia: c cercus, epi epiphallus, go bas gonocoxa basistylus, go dis gonocoxa dististylus, par paraprocts, pm paramere, p penis, pe penunci, spa superanale, sV-sIX fifth to ninth sterna, and tVI-tIX, sixth to ninth terga.

**Nomenclatural acts**. This published work and the nomenclatural acts it contains have been registered in ZooBank, the proposed online registration system for the International Code of Zoological Nomenclature (ICZN). The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSIDs for this publication are urn:lsid:zoobank.org:pub:8E7D07F9-A618-48D6-8EEC-F5AC68593C5C (for publication); urn:lsid:zoobank.org:act:A219BB2D-209F-4D2E-ABAA-AB10CB8CF0D8 (for Dualulidae fam. nov.); urn:lsid:zoobank.org:act:E9F85E03-B6C6-41FF-A82D-16EE9CFC21C0 (for *Dualula* gen. nov.); urn:lsid:zoobank.org:act:70C21743-5FEF-48C1-8FE4-7739BC029394 (for *Dualula kachinensis* sp. nov.).

**Reporting summary**. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information Files. Higher-resolution versions of the figures (https://doi.org/10.6084/m9.figshare.7775801.v1) and supplementary data (https://doi.org/10.6084/m9.figshare.7775822.v1) have been deposited in the figshare database. All relevant data are available from the corresponding authors upon request.

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## Author contributions

D.R. and C.C.L. designed the experiments. X.D.L., C.K.S., and D.R. contributed materials and analytical tools. X.D.L. took the photographs. X.D.L. and C.C.L. made the line drawings and reconstruction. X.D.L., C.C.L., and C.L.H. were responsible for palynology and entomophily inferences. X.D.L., C.C.L., C.K.S., C.L.H., and D.R. performed the analyses, experiments and wrote the manuscript. All authors read and approved the final manuscript.

## Additional information

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## Supplementary Information

# Life habits and evolutionary biology of new two-winged long-proboscid scorpionflies from Mid-Cretaceous Myanmar amber

Lin et al.

**Supplementary Note 1 │** Phylogeny and Systematics of Mecoptera and Establishment of Dualulidae as a New Family……………………………………………………………………... 2
Historical Context…………………………………………………………………………........................................ 2
Phylogenetic Procedures and Methods…………………………………………..…………………………………… 3
Phylogenetic Results…………………………………………………………………..…………………...................... 3
**Supplementary Figure 1│**Comparisons of eight, historically different results of phylogenetic analyses showing relationships among major holometabolan clades……………………………… 4
**Supplementary Figure 2 │**Illustrations of the paratype of *Dualula kachinensis* gen. et sp. nov. ……………………………………………………………………………………............... 5
**Supplementary Figure 3 │** Details of the proboscis, calypter and genitalia for paratype *Dualula kachinensis* gen. et sp. nov. ………………………………………………………………….. 6
**Supplementary Figure 4 │** The paratype of *Dualula kachinensis* gen. et sp. nov. ……..……………………….. 7
Description of *Dualula kachinensis* Lin, Shih, Labandeira and Ren gen. et sp. nov…………………………………………………………………………………………………………… 8
**Supplementary Figure 5 │** Proboscis and genitalic details of paratype *Dualula kachinensis* gen. et sp. nov. ……………………………………………………………………………………... 8
Evolution of Wing Venation in Mid-Mesozoic Scorpionflies…………………………………………………… 10
**Supplementary Figure 6 │** Comparison of forewing venation in *Vitimopsyche, Lichnomesopsyche* and *Dualula*……………………………………………………………………………………….. 11
Future Studies of Pseudopolycentropodidae, *Parapolycentropus* and Dualidae…………………………………… 13

**Supplementary Note 2 │** Mouthparts of Long-Proboscid Mecoptera……………….…………………… 14

**Supplementary Note 3 │** *Dualula* Mouthpart Structure………………………………………….................. 17
**Supplementary Figure 7 │** Proboscis details of *Pseudopolycentropus janeannae*…………………………….. 17
**Supplementary Figure 8 │** Details of the proboscis base and other mouthpart elements of *Parapolycentropus* spp……………………………………………………………………………… 18
**Supplementary Figure 9 │** Proboscis of *Parapolycentropus paraburmiticus*…………………………………… 20

**Supplementary Note 4 │** Feeding Processes and Food Sources of *Parapolycentropus* and Dualulidae………………………….......................................................................... 20
Associations with Gymnosperms……………………………………………………………………………………… 21
**Supplementary Figure 10 │**Measurements and distribution of *Cycadopites* sp. pollen grains adjacent to *Parapolycentropus paraburmiticus*……………………………………………………… 22
Possible Associations with Angiosperms……………………………………………………………..……….. 22
**Supplementary Figure 11 │** *Tropidogyne* spp. and five angiosperm flower morphotypes with attached stamens from Myanmar amber…………..……………….................................. 23

**Supplementary Note 5 │** Hind-Wing Reduction in Mid-Mesozoic Insects, Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae…………………………………………… 24

**Supplementary Note 6 │** Genitalia Structure in Mesopsychidae, Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae…………………………………………… 26
**Supplementary Figure 12 │** Male genitalia of *Pseudopolycentropus janeannae*……………………………… 26
**Supplementary Figure 13 │** Male genitalia of *Lichnomesopsyche daohugouensis*…………………………..…. 27
**Supplementary Figure 14 │** Male genitalia of *Epicharmesopsyche pentavenulosa*…………………………..….. 28

**Supplementary Note 7 │** Reproductive Biology of *Parapolycentropus* and Dualulidae…………………………………………………………………………………………………… 29
**Supplementary Figure 15 │** Three entombed swarms of *Parapolycentropus* spp…………………………….. 29
**Supplementary Figure 16 │** Copulating *Parapolycentropus paraburmiticus*……………………………........... 30

**Supplementary Table 1**……………………………………………………………………………...……….. 33

**Supplementary References**……………………………………………………………………………………… 34

## Supplementary Note 1 │ Phylogeny and Systematics of Mecoptera and Establishment of Dualulidae as a New Family

### Historical Context

During the past half century, numerous phylogenetic analyses that have attempted to resolve the phylogenetic relationships among the major lineages within Holometabola[1–22]. Hennig, in his seminal work in 1969, presented a phylogeny of Holometabola that included Neuroptera and Mecopteroidea, the latter of which involved an investigation of the relationships among the mecopteroid groups of Mecoptera, Diptera and Siphonaptera, and their phylogenetic connection to Amphiesmenoptera (Trichoptera + Lepidoptera)[1]. At the time these analyses were based entirely on morphology; with character matrix construction and character sampling often incomplete, notably in Hennig's[1] and Boudreaux's[2] studies. Nevertheless, their efforts had substantive implications for later research. For example, Hennig placed Mecoptera and Diptera as a sister-group to Amphiesmenoptera, and Siphonaptera was phylogenetically close to Neuroptera and distant from Mecoptera[1] (Supplementary Fig. 1a). The study of Kristensen (1999)[3] partly supported the Hennig's earlier results[1]. Nevertheless, Boudreaux provided evidence for Siphonaptera as the sister-group to Diptera, and together with Mecoptera, formed a monophyletic group[2] (Supplementary Fig. 1b).

With the development of molecular phylogenetic approaches, molecular character matrices supplanted morphological data and became the mainstay for analyses of Holometabola, especially for those analyses that only contained extant groups. Later, phylogenetic analysis typically involved a total evidence approach that included morphological and molecular data, such as analyses by Beutel and colleagues[4], which provided a result similar to that of Boudreaux[2]. In two other analysis employing a total evidence approach, Diptera was proposed as allied with Amphiesmenoptera, and Mecoptera was a sister-taxon of Siphonaptera[5,6] (Supplementary Fig. 1g). These two analyses were based on 18S ribosomal RNA[5] and mitochondrial genome[6] data, respectively. By contrast, Chalwatzis and colleagues proposed that Diptera derived from within a clade consisting of Mecoptera, Siphonaptera and Neuropteroidea, and that Amphiesmenoptera was the basal member in these three groups[7] (Supplementary Fig. 1d). This phylogenetic topology[7] was at variance with views that are more recent.

Whiting was the first to attempt a phylogenetic analysis of extant Mecoptera with multiple gene data, Based on his results[8] (Supplementary Fig. 1f), Mecoptera is a paraphyletic group, with Boreidae the sister-group of Siphonaptera, and the position of Diptera lay outside of Mecoptera and Siphonaptera. As Whiting's data[8] included only extant groups, his phylogeny resulted in gaps that lacked fossil taxa. Similarly, the subsequent analysis by Song et al.[6], which used mitochondrial genome data for their phylogenetic analysis of Holometabola, confirmed that mitochondrial genome data was insufficient for deciphering deep relationships within Holometabola[6]. Currently, most results have (i) supported Mecoptera and Siphonaptera as forming a clade that has a close relationship to Diptera; (ii) determined that Amphiesmenoptera is a monophyletic group; and (iii) relegated Neuroptera as a significantly distant group from Mecoptera, Siphonaptera, Diptera, Trichoptera and Lepidoptera (Supplementary Fig. 1c). Of these studies, some contain only morphological matrices[9,10], others molecular matrices[11–16], and more recently total evidence[17–19]. However, the majority of these analyses has focused only on extant groups[20], and consequently has ignored the importance of fossil records. Such a focus has resulted in the lack of exploration in the relationship between extinct and extant groups. By integrating extinct and extant groups into a phylogenetic analysis[21], the paraphyly of Mecoptera is upheld, rendering Diptera and Siphonaptera as lineages within Mecoptera and the existence of a close relationship between Amphiesmenoptera and the most basal Mecoptera lineage, Kaltanidae[21] (Supplementary Fig. 1h).

The results of our research (Fig. 1; Supplementary Fig. 1i) indicate Amphiesmenoptera and Thaumatomeropidae are sister groups, and basal Diptera and Siphonate are close to the new clade

Dualulidae and *Parapolycentropus*. Based on our analysis, we conclude that the subgroups of Antliophora – Mecoptera, Diptera and Siphonaptera – are paraphyletic. This result generally is similar to the conclusion of Wheeler et al.[20] (Supplementary Fig. 1e), but differs from other studies, including Ren et al.[21], that result in several clades. Our new results are different in four ways. First, basal Diptera and Siphonaptera occur within Mecoptera, rather than the sister group to Mecoptera. Second, Meropeidae and Eomeropidae are more basal than other extant families, compared to the basalmost position of Nannochoristidae in the previous studies. Third, basal Diptera has a close relationship with Siphonaptera. Fourth, Amphiesmenoptera and Thaumatomeropidae are sister-groups.

## Phylogenetic Procedures and Methods

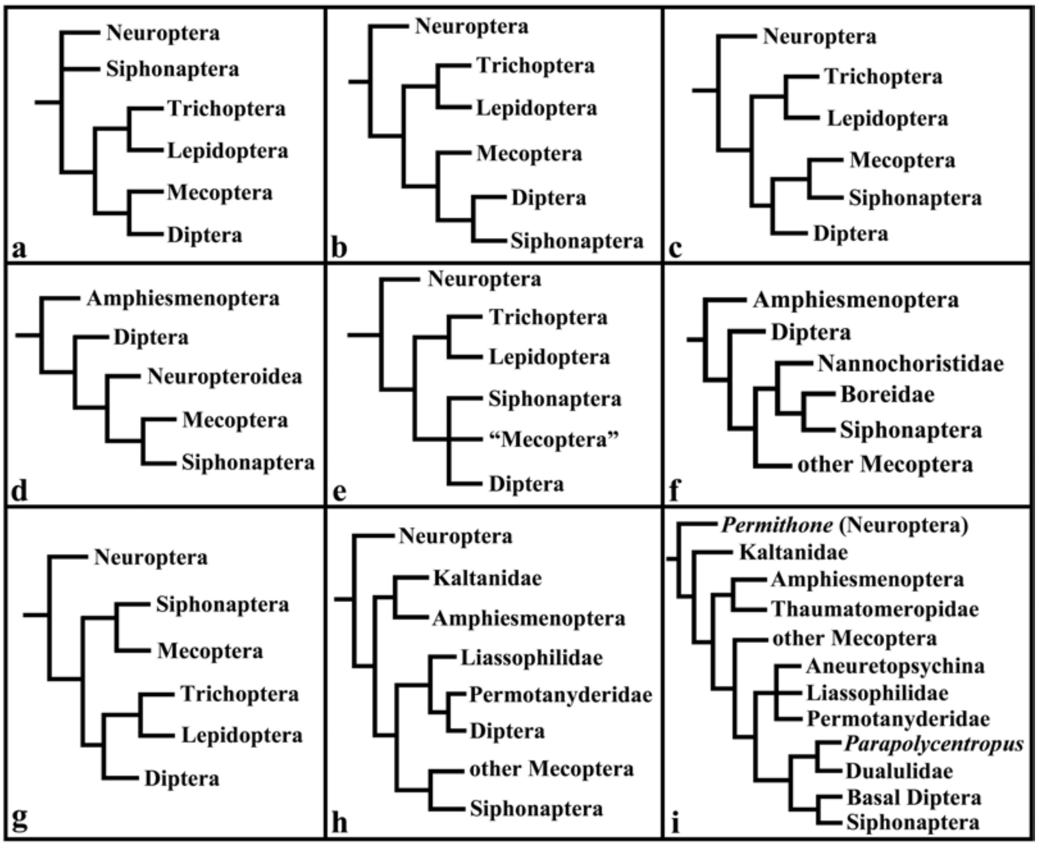
We carried out a phylogenetic analysis to explore the taxonomic position of Dualulidae and to clarify the phylogenetic relationships between the two-winged mecopteran taxa and other scorpionflies, including the long-proboscid clade Aneuretopsychina. Because of an absence of well-preserved body features in most specimens of the extinct families, we were able to include in the analysis only some of the available body characters. The list of characters (Supplementary Data 1) included features of the mouthparts, head, thorax and legs (characters 0–13); features of the wings such as vein number, wing shape and venation (characters 14–46); and features of the abdomen and genitalia (characters 47–50). All 51 characters were used in the phylogenetic analysis. The character selection was partly attributable to the characters used in the phylogenetic analyses of Ren et al. (2009)[21] and Lin et al. (2016)[22]. Thirty-four genera or subfamilies comprising six extant families of Mecoptera, Siphonaptera, and seven genera of basal Diptera with complete or nearly complete preserved wings and bodies were selected as ingroups for the analyses (Fig. 1).

Type genera were used for most families of Mecoptera in the analyses. However, if the type genera for some families lacked a full or nearly full complement of features, such as an incomplete fore- or hind wing, we employed non-type genera that were more complete morphologically to represent the full or fullest character set achievable for the given taxon. For example, the family Aneuretopsychidae is best represented not by *Aneuretopsyche*, but rather *Jeholopsyche*, the only genus with well-preserved body and mouthpart features for this family. For basal Diptera, selection of genera originated from the tree of Blagoderov et al. (2007)[23]. The earliest fossil records of siphonate proboscides – *Permithone* as Permithonidae)[24–26] and Amphiesmenoptera[1,13,27,28] – were selected as outgroups. The selection of outgroups was based on the phylogenetic results of the Misof et al. (2014) study[13] and the analysis of Wiegmann et al. (2009)[14] that showed relationships among dipterans, siphonapterans, mecopterans and related taxa, including stem-groups. We chose the type genus *Permithone* of Permithonidae (Neuroptera) as the root of the tree, and a character-state matrix of 37 taxa and 51 morphological characters with two or more character states (Supplementary Data 2).

The character-state matrix was entered into WinClada (Version1.00.08)[29]. For a tree search we used a heuristic search method, with options set to hold 10,000 trees, 1000 replications, 100 starting tree replications, and a multiple TBR + TBR search strategy. All characters were treated as unordered and equally weighted. Missing characters were coded with a question mark and inapplicable characters with an em dash. Multi-state characters were denoted as a “0+1” mark. Parsimony analyses were performed by NONA (Version 2.0)[30] using an exhaustive search option and bootstrap support values from 1000 replications are presented as numbers under branch nodes.

## Phylogenetic Results

All trees indicate that Mecoptera are a paraphyletic group, and the two most basal taxa are Thaumatomeropidae[22] consisting of *Thaumatomerope*[31,32] and Kaltanidae[33] represented by *Altajopanorpa*[33,34]. Thaumatomeropidae exhibits a close relationship to Amphiesmenoptera (Fig. 1a), but,



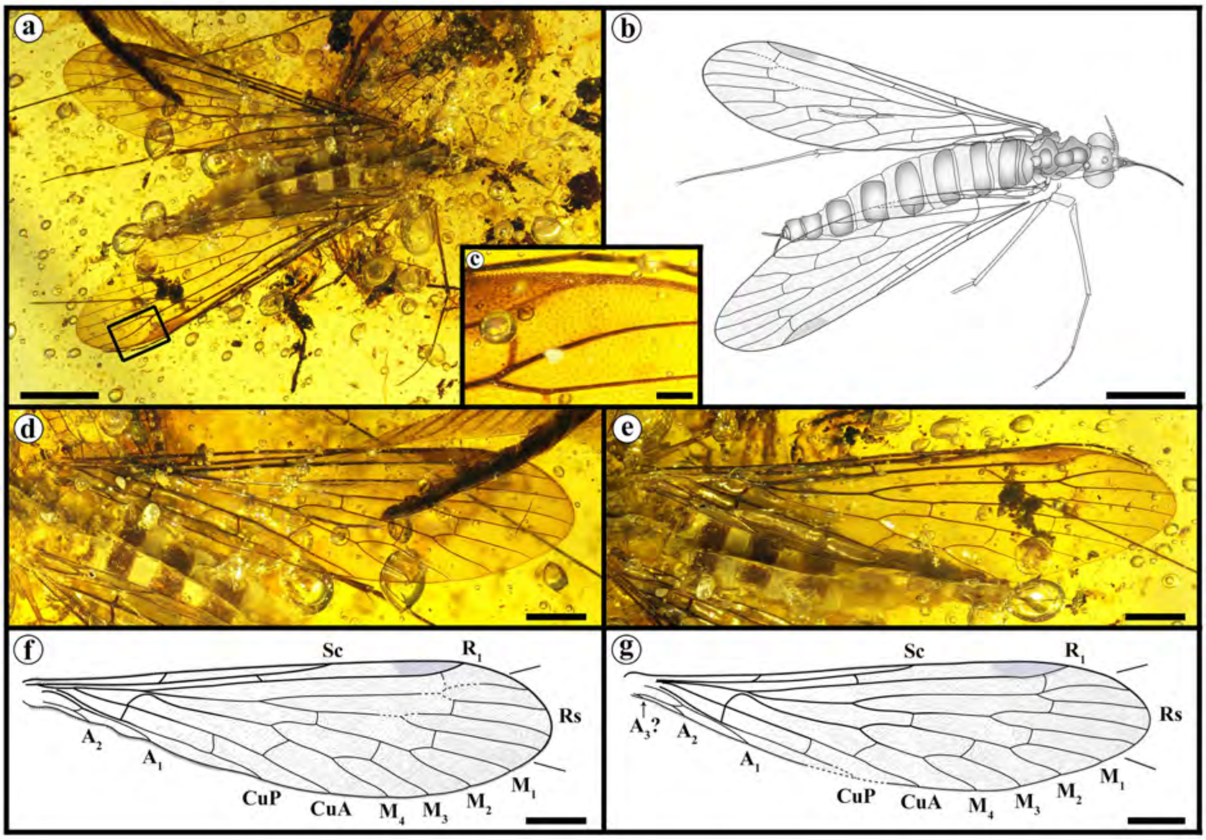
**Supplementary Figure 1 │Comparisons of eight, historically different results of phylogenetic analyses showing relationships among major holometabolan clades**. The included clades are Neuroptera, Amphiesmenoptera (Trichoptera, Lepidoptera), and Antliophora (Mecoptera, Diptera, Siphonaptera) and subgroups. (**a**), Phylogenetic tree abstracted from Hennig (1969)[1]. (**b**), Phylogenetic tree abstracted from Boudreaux (1979)[2]. (**c**), Phylogenetic tree abstracted from Misof et al. (2014)[13] and Wiegmann et al. (2009)[14]. (**d**), Phylogenetic tree abstracted from Chalwatzis et al. (1996)[7]. (**e**), Phylogenetic tree abstracted from Wheeler et al. (2001)[20]. (**f**), Phylogenetic tree abstracted from Whiting (2002)[8]. (**g**), Phylogenetic tree abstracted from Song et al. (2016)[6]. (**h**), Phylogenetic tree abstracted from Ren et al. (2009)[21]. (**i**), Phylogenetic tree abstracted from Fig. 1 of this study.

this link is established only on two homologous characters, defined by fewer crossveins (Character 41:1) and lack of pterostigma in the forewings (Character 43:1). A revision of these taxa would be in order with use of additional, better-preserved fossil material than at present. These two nominal mecopteran groups, with Amphiesmenoptera, serve as the sister group of all other Mecoptera, though the basal position of Thaumatomeropidae and Kaltanidae is not secure. Meropeidae[35] consists of *Burmomerope*[36] and Eomeropidae, with *Eomerope* as the basalmost group of extant families[36,37]. Eomeropidae shares a sister-group relationship to other Mecoptera, basal Diptera, and Siphonaptera; however, this clade is supported by only one synapomorphic character, defined as leg pubescence forming regular, at least local encirclement features (Character 12:1). Nevertheless, the oldest fossil records of these two families are from the late Middle to early Late Triassic of Kyrgyzstan[32,38], a relatively late date when compared to the Late Permian for the oldest Aneuretopsychina[11,39,40]. This discrepancy in age may be attributable to the rarity of adequate fossil preservation or absence of discovery.

Two features render Belmontiidae, consisting of *Belmontia*[41] and its sister group, the balance of the clade, as forming a single lineage. One of these features is the unambiguous character of the presence of less than four anterior branches of the Sc vein in the forewing (Character 21:1); the other is the number of vein branches of the MP vein, a homologous character (Character 30:1). From this relationship, the Permochoristinae[42] – equivalent to Permochoristidae[42,43] – was present during the late Permian, together with Sibiriothaumatidae that consisted of *Sibiriothauma*[44]. Previously, the earliest fossil record for *Sibiriothauma* was from the Late Jurassic of Russia[44]. *Sibiriothauma* forms a distinct clade with our data, confirming the homologous nature of the number of Rs branches and the relative level along the wing of the A2 vein ending versus the origination of the MP vein from the CuA vein in the forewings (Characters 26:1 and 39:1). This clade comprises the remainder of mecopteran taxa, seven basal dipteran lineages and Siphonaptera that are validated by three synapomorphic characters (Characters 22:1, 23:1 and 49:2) and one homologous character (Character 25:0). Characters 23 and 49 have parallelisms and reversals in the remaining branches. Parachoristidae[45] and the other four families of Panorpoidea display paraphyly. The other groups of this clade are monophyletic, supported by two synapomorphic features, a relatively short Sc vein in the forewings (Character 20:1) and hind wings (Character 44:1). There also are four homologous characters (Characters 19:1, 29:0, 41:1 and 49:1).

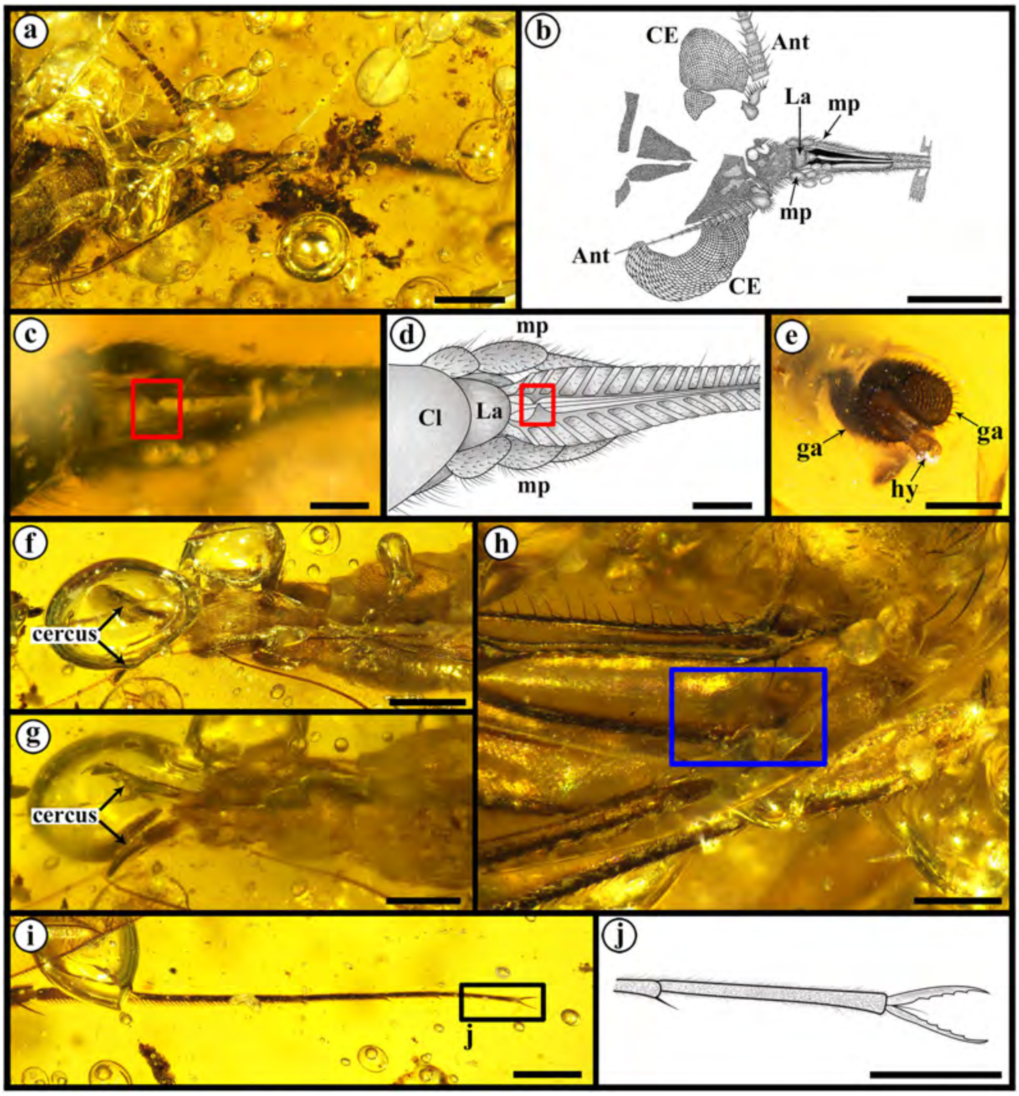
Curiously, six families of modern Mecoptera lack close relationships and occur with extinct families represented in the middle region of the trees (Fig. 1). These extant lineages evidently originated during the mid Mesozoic. Nannochoristidae[42] currently is recognized as the basalmost extant lineage[8], and has a sister-group relationship with extant Bittacidae[35] and extinct Robinjohniidae[46]. This relationship is supported by one synapomorphic character, the long sub-basal fusion of the MP and CuA veins in the forewings (Character 33:1). Two homologous characters (Characters 32:1 and 47:1) occur as well.

Toward the terminus of the tree, Aneuretopsychina are established as a paraphyletic group that includes Liassophilidae and Permotanyderidae. Previously, Aneuretopsychina was considered a single,

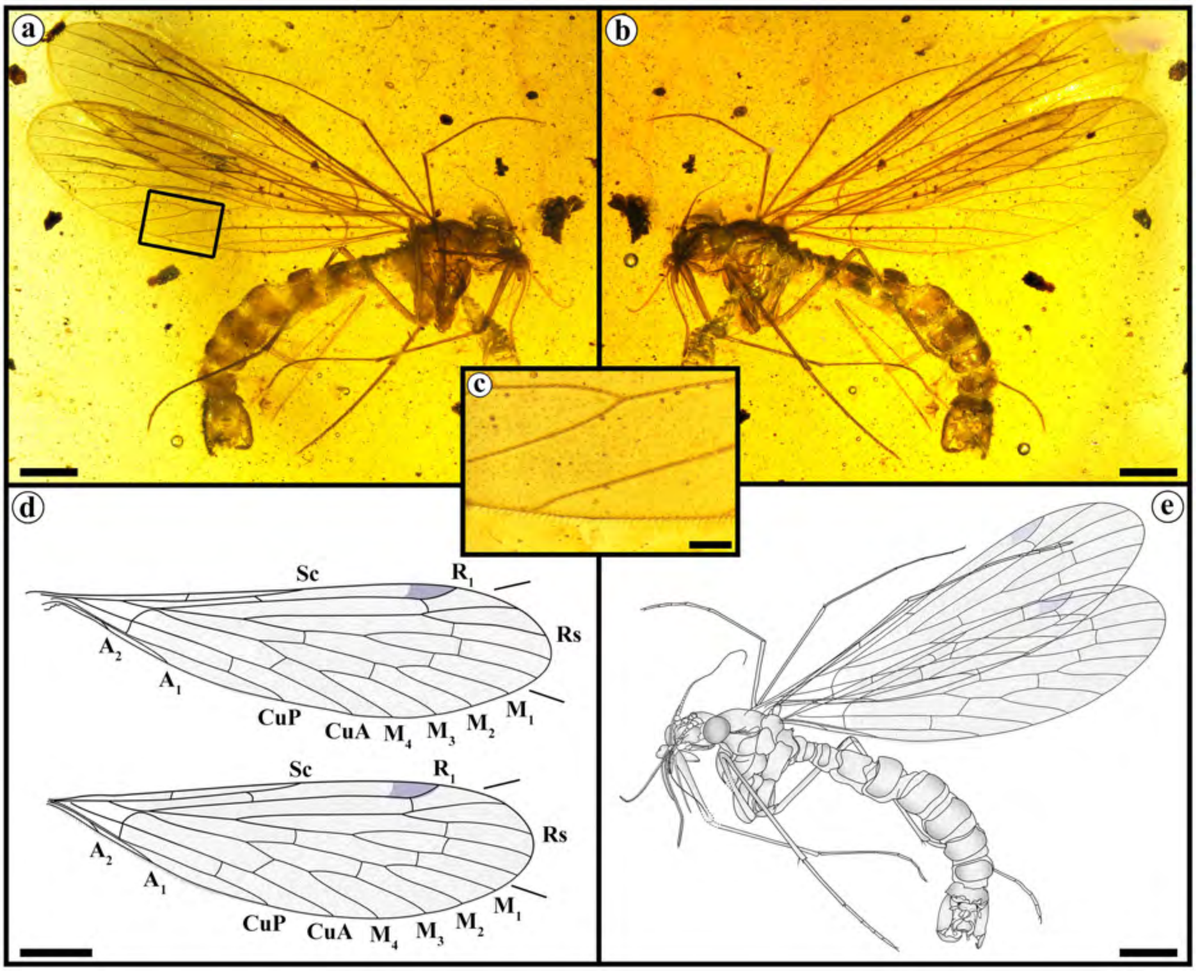


**Supplementary Figure 2 │ Illustrations of the paratype of *Dualula kachinensis* gen. et sp. nov.** This specimen is CNU-MEC-MA-2017016, a female. (**a**), Paratype in dorsal view. (**b**), Overlay drawing of the paratype in dorsal view. (**c**), Details of marginal setae and membrane of the right forewing. (**d**), Left forewing. (**e**), Right forewing. (**f**), Line drawing of left forewing. (**g**), Line drawing of right forewing. Scale bars represent 2 mm in (a) and (b), 1 mm in (d)–(g), and 0.2 mm in (c).

independent clade[21], buttressed by two explicit characters, siphonate mouthparts (Character 4:1), and maxillary palpi that were short and adpressed to the base of the proboscis or alternatively lost as a multi-article structure (Character 5:1). However, the absence of complete body features of known Liassophilidae[47] and Permotanyderidae[26], especially involving presence or absence of a proboscis and other mouthpart elements, is an issue. The presence of a proboscis in these two lineages may expand the monophyly of the three genera currently constituting Pseudopolycentropodidae[48,49] (Supplementary Table 1), presently established only by three homologous features (Characters 12:2, 25:0 and 46:0). These three features yield the same result for the other three families of Aneuretopsychina (Characters 15:0, 16:0 and 32:1) consisting of Dualulidae, *Parapolycentropus*[50], and Siphonaptera.



**Supplementary Figure 3 │ Details of the proboscis, calypter and genitalia for paratype *Dualula kachinensis* gen. et sp. nov.** This specimen is CNU-MEC-MA-2017016, a female. (**a**), Head and proboscis in dorsal view. (**b**), Combined camera lucida and overlay drawing of the head and mouthparts; the ovoidal and circular structures are bubbles that occlude structural details. (**c**), Proboscis base. (**d**), Line drawing of proboscis base. (**e**), Proboscis cross-section – revealing outer galeal food tube and inner hypopharyngeal salivary duct – that is uniquely exposed at the amber surface. (**f**), Female genitalia in dorsal view. (**g**), Female genitalia in ventral view. (**h**), Right hind wing (calypter) in dorsal view. (**i**), Left hind leg in dorsal view. (**j**), Line drawing of telotarsus and claws of left hind leg. Red rectangles in (c) and (d) indicate the distinctive valve regulating the salivarium chamber at the hypopharyngeal base. Scale bars represent 0.5 mm in (a), (b), (f), (g) and (i); 0.1 mm in (c)–(e); and 0.2 mm in (h) and (j).



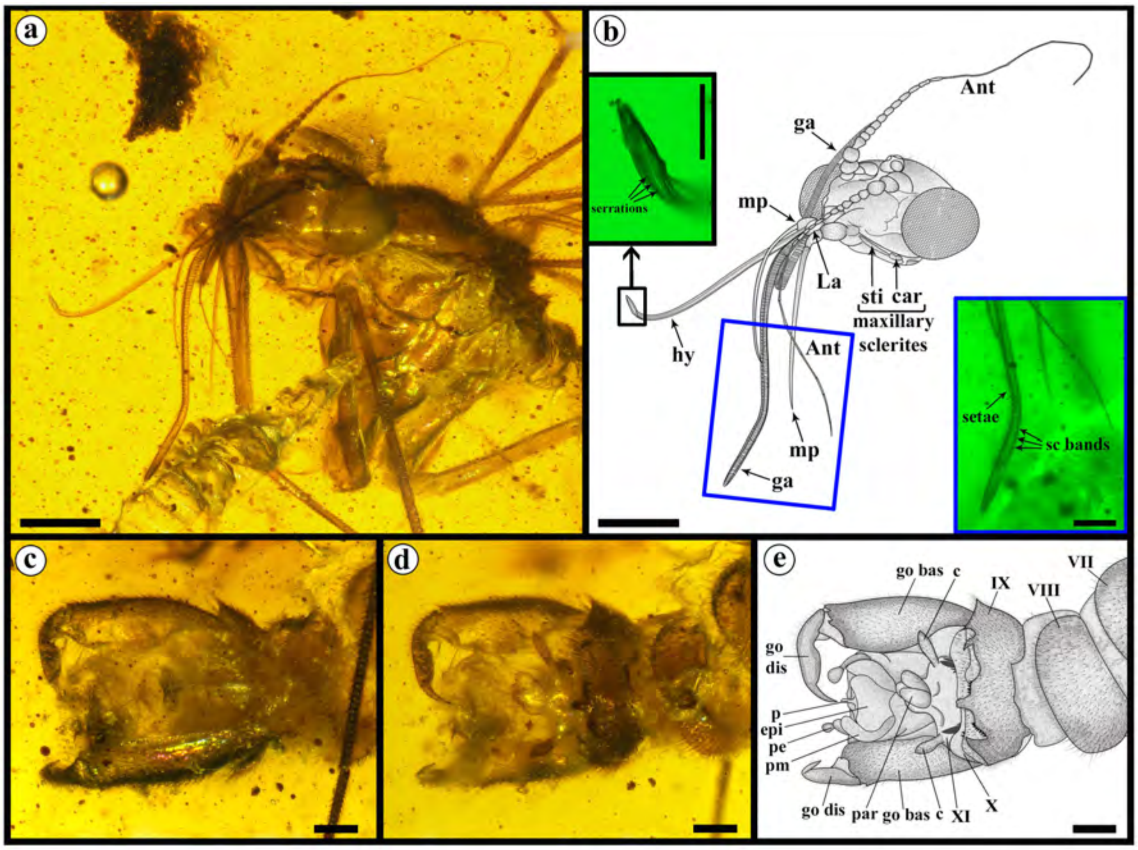
**Supplementary Figure 4 │ The paratype of *Dualula kachinensis* gen. et sp. nov.** This specimen is CNU-MEC-MA-2017017, a male. (**a**), Paratype in right lateral view. (**b**), Paratype in left lateral view. (**c**), Details of setae on the left forewing membrane margin. (**d**), Line drawing of forewings; above is the right and below is the left forewing. (**e**), Overlay drawing of (b). Scale bars: 1 mm in (a), (b), (d) and (e); 0.2 mm in (c).

Seven basal dipteran taxa constitute a clade that is supported by five synapomorphic characters. The first character is that only the mesothorax is structurally robust or alternatively none of the three thoracic segments is structurally robust (Character 8:1). Second, only one pair of wings is present or alternatively both pairs are lost (Character 14:1).Third, heteronomous hind wings are reduced in size and modified into halteres or haltere-like structures (Character 15:2). Fourth, thickened setae are present along the margin of the forewing (Character 18:1). Fifth, the hind wings are vestigial (Character 45:1). Additionally, two homologous features (Characters 11:1 and 49:0) are present. Three definite features support the clade of basal Diptera and Siphonaptera. They are i), absence of a membranous area between the mesopleura and metapleura (Character 10:1); ii), partial axillary sclerites occurring at the base of the forewings (Character 17:1); and iii), hind wings are modified into halteres (Character 45:2). By contrast, seven genera of basal dipterans and Siphonaptera are not adequately separated and display extensive paraphyly. Due to the wingless condition of Siphonaptera, most characters used in our analysis are not applicable. Therefore, the position of Siphonaptera cannot be well- established. The resulting tree 24 (Fig. 1b) essentially is the same as the strict consensus tree (Fig. 1a), with two obvious differences. The first difference is that Parachoristidae[45] and the four families of Panorpoidea – Dinopanorpidae[51], Orthophlebiidae[35], Panorpidae[52] and Panorpodidae[48] – show collapse of three nodes resulting in these families becoming paraphyletic in the consensus tree (Fig. 1a). Second, there are no synapomorphic characters distinguishing the basal dipterans and Siphonaptera, resulting in extensive paraphyly in the consensus tree. In addition, only one synapomorphic character (Character 10:1) supports this clade.

## Description of *Dualula kachinensis* Lin, Shih, Labandeira and Ren, gen. et sp. nov.

This description is based mostly on the holotype specimen, but also is partly based on two paratype specimens that especially involve details of the head, proboscis and genitalia. (See Figs. 1–3 and Supplementary Figs. 2–5).

Head and mouthparts. Head triangular in dorsal view; mouthparts prognathous. Three ocelli present, and several long and robust setae on the vertex (Fig. 1i; Supplementary Fig. 5a,b). Distinctive carinae between clypeus and antennae. Antenna filiform, with many articles; scape tubular and large; pedicel slightly funnel-shaped; flagellum with 20 flagellomeres, the basal 10 flagellomeres much thicker and shorter than the remaining 10, among them flagellomeres 1–6 larger than flagellomeres 7–10; flagellomeres 11–20 long and thin, almost long rectangular; and last two flagellomeres much shorter than flagellomeres 11–18. Compound eyes large, oval and widely separated. Proboscis long and narrow; (Fig. 1a,b,d,i; Supplementary Fig. 5a,b); male shorter than female (Supplementary Fig. 2a,b); lacking apical pseudolabellae, covered with dense setae or smaller microtrichia and displaying encircling annuli or bands. Labrum relatively small, triangular and apex slightly round. Proboscis divided into three elements:



**Supplementary Figure 5 │ Proboscis and genitalic details of paratype *Dualula kachinensis* gen. et sp. nov.** This specimen is CNU-MEC-MA-2017017, a male. (**a**), Head and proboscis in left lateral view. (**b**), Line drawing of head and proboscis in (a), and enlargement of hypopharynx tip (upper left) and galeae (lower right) under green epifluorescence. (**c**), Male genitalia in ventral view. (**d**), Male genitalia in dorsal view. (**e**), Line drawing of male genitalia in (d). Abbreviations: **car**, cardo; **ga**, galea; **hy**, hypopharynx; **mp**, maxillary palp; **sti**, stipes; **c**, cercus; **epi**, epithallus; **go bas**, gonocoxa basistylus; **go dis**, gonocoxa dististylus; **p**, penis; **par**, paraprocts; **pe**, penunci; **pm**, paramere; and **tVI**–**tIX**, sixth to ninth terga. Scale bars represent 0.5 mm in (a) and (b); 0.1 mm in hypopharynx tip of (b), 0.2 mm in galeae tip of (b); and 0.2 mm in (c)–(e).

a hypopharynx and a pair of encompassing galeae. Maxillary palp originating from palpifer of stipes connected to cardo; these maxillary sclerites located on either side of central long, labial sclerite, the mentum. Maxillary palp with three articles, the second article widest and terminal article much longer and narrower than other two, especially extended in male with length ca. two-thirds of galea and hypopharynx (Supplementary Fig. 5a,b). Each galea a half tube in lateral section (Supplementary Fig. 3e), with sclerotized and setose ring bands on outer surface, but inner-surface smooth and lacking setae; galeae sutured to each other, forming a food channel that encompasses the hypopharyngeal salivary duct. Salivary duct deployed as a narrow tube emerging from a valved salivarium chamber (hypopharyngeal pump) under labrum (Supplementary Fig. 3a–d), positioned rectilinearly from labrum to proboscis tip (Fig. 1g–i; Supplementary Figs. 3c–e; 5a,b); many serrations present on ventral aspect of salivary duct (Fig. 1g,h; Supplementary Figs. 3e, 5b). Galea more flexible than hypopharynx; both elements not bound to each other (Fig. 1g; Supplementary Fig. 5b).

Thorax and Legs. Pronotum and metanotum small and similar to each other; mesonotum relatively large (Fig. 2a,b). Scutum and scutellum distinct on mesonotum and metanotum. Legs slender and entirely covered with pubescence (Supplementary Fig. 3i,j) but not arranged into annulus. Femora elongate; tibiae long and slender, with at least one apical spur. Tarsi of five segments; the first segment longest and nearly the same length as rest of segments, last two segments shortest and same in size and shape; pretarsus with two claws and one long bristle between them, the inner side of each claw bears four sharp tooth-like structures, all similar in size and shape (Supplementary Fig. 3i,j).

Wings. Left forewing slender, right forewing very similar to left forewing (Fig. 1a–e; Supplementary Figs. 2, 4). Membrane delicate and tegula small (Fig. 2c–f). Sc relatively short; $R_1$ single and straight at the base, proximal to $Rs_{1+2}$ bifurcation, slightly curved near the margin; one sc-$r_1$ crossvein present, at the same level of or distal to Rs bifurcation. Pterostigma well preserved. Both Rs and M with four branches; $Rs_{1+2}$ bifurcation distal to $Rs_{3+4}$ bifurcation; one crossvein between $R_1$ and $Rs_1$, $Rs_{1+2}$ and $Rs_{3+4}$ respectively, and one $rs_3$-$rs_4$ crossvein present in male (Supplementary Fig. 4d); $r_1$-$rs_1$ slightly distal to $Rs_{1+2}$ bifurcation, $rs_2$-$rs_3$ distal to $rs_4$-$m_1$; R from Sc proximal to M from CuA. M with four long branches, $M_{1+2}$ forking distal to $M_{3+4}$ bifurcation and much distal to $Rs_{3+4}$ bifurcation, but proximal to $Rs_{1+2}$; one crossvein between $M_{1+2}$ and $M_3$, very distal to $M_{3+4}$; M originating from CuA proximal to Rs from $R_1$; CuA, CuP. $A_1$ and $A_2$ single; $A_1$ relatively long and slightly distal to $Sc_1$, $A_2$ short; two crossveins between CuA and CuP, and one between $A_1$ and $A_2$. One calypter at the base of each forewing, with some thick, stiff setae along the margin of the apex (Fig. 2c–f; Supplementary Fig. 3h). Hind wing reduced to a minute, tubular-shaped lobe, with vestiges of some wing venation; relatively smooth, the length nearly the half of forewing calypter; also included an anterior lobe above the hind wing, and likely comprised of a tegula (Fig. 2c–e).

Abdomen and genitalia. In the female, abdomen elongate and tapering apically, with 11 segments (Supplementary Fig. 2a,b). T1 partly fused with metathorax, segments 8 and 9 much smaller than segments 2–7. The last two segments (10 and 11) very small and closely combined with each other. Tergites large and typical of scorpionflies, but sternites unknown due to preservation in both holotype and paratypes. Cercus originating from the eleventh segment and with two obvious articles, the first article slightly longer and thicker than second one, all articles covered with short and thick setae (Fig. 1f; Supplementary Fig. 3f,g).

In the male, abdomen much more slender than female, with nine visible segments. T1 small and fused somewhat with metathorax; the first and second segments much smaller than others. T9 enlarged, partly covering segments 10 and 11. Tergites wide, but sternites reduced and not visible because of obscuring light color (Supplementary Fig. 4a,b,e). Male genitalia well preserved; tergites 10 and 11 relatively small; a pair of short cerci and an anal orifice originating from segment 11 (Supplementary Fig.

5d,e); paraprocts almost entirely covering the anal orifice. The ninth sternite (S9) enlarged and occupying nearly entire venter of gonosomite; clasper (or gonocoxa) large and robust, originating from sternite 9 and divided into two distinct segments – basistylus and dististylus. Basistylus immovable and combined with S9; dististylus hook-shaped and somewhat expanded in its middle section but tapering, length about two thirds of basistylus. Aedeagus consists of three parts, epiphallus, penunci and paramere, and penis; epiphallus infundibulate and almost covers the penis; parameres slender and rodlike, but penunci much smaller and tubular (Supplementary Fig. 5d,e). Several long and thick setae or bristles cover the ninth tergum tIX, paraprocts, cercus and clasper (Supplementary Fig. 5c–e).

## Evolution of Wing Venation in Mid-Mesozoic Scorpionflies

In Neuroptera, three functionally two-winged species were described from the Cretaceous, potentially providing additional insight into the evolutionary process of wing reduction. A mantispid species, *Mantispidiptera enigmatica* (Mantispidae), from Upper Cretaceous (Turonian) amber of New Jersey[53], displays a two-winged condition with highly reduced hind wings. Similarly, the two-winged lacewing, *Dipteromantispa brevisubcosta* (Dipteromantispidae), was described from the Lower Cretaceous (latest Barremian–earliest Aptian) Yixian Formation of Liaoning, in Northeastern China[54]. This specimen displayed two normal forewings while hind wings were modified into small halteres. Lastly, the species *Pedanoptera arachnophila* (Mesochrysopidae) was described from Myanmar amber[55]. This species also exhibited highly reduced hind wings, but unlike the other taxa, it retained a few discernable longitudinal veins[55]. Mid-Mesozoic hind-wing reduction also independently appeared in several lineages of Mecoptera. Such hind-wing specialization is not an accidental phenomenon, but rather represents a cascade of evolutionary developmental processes that was repeated in several, major insect lineages such as Neuroptera and Mecoptera.

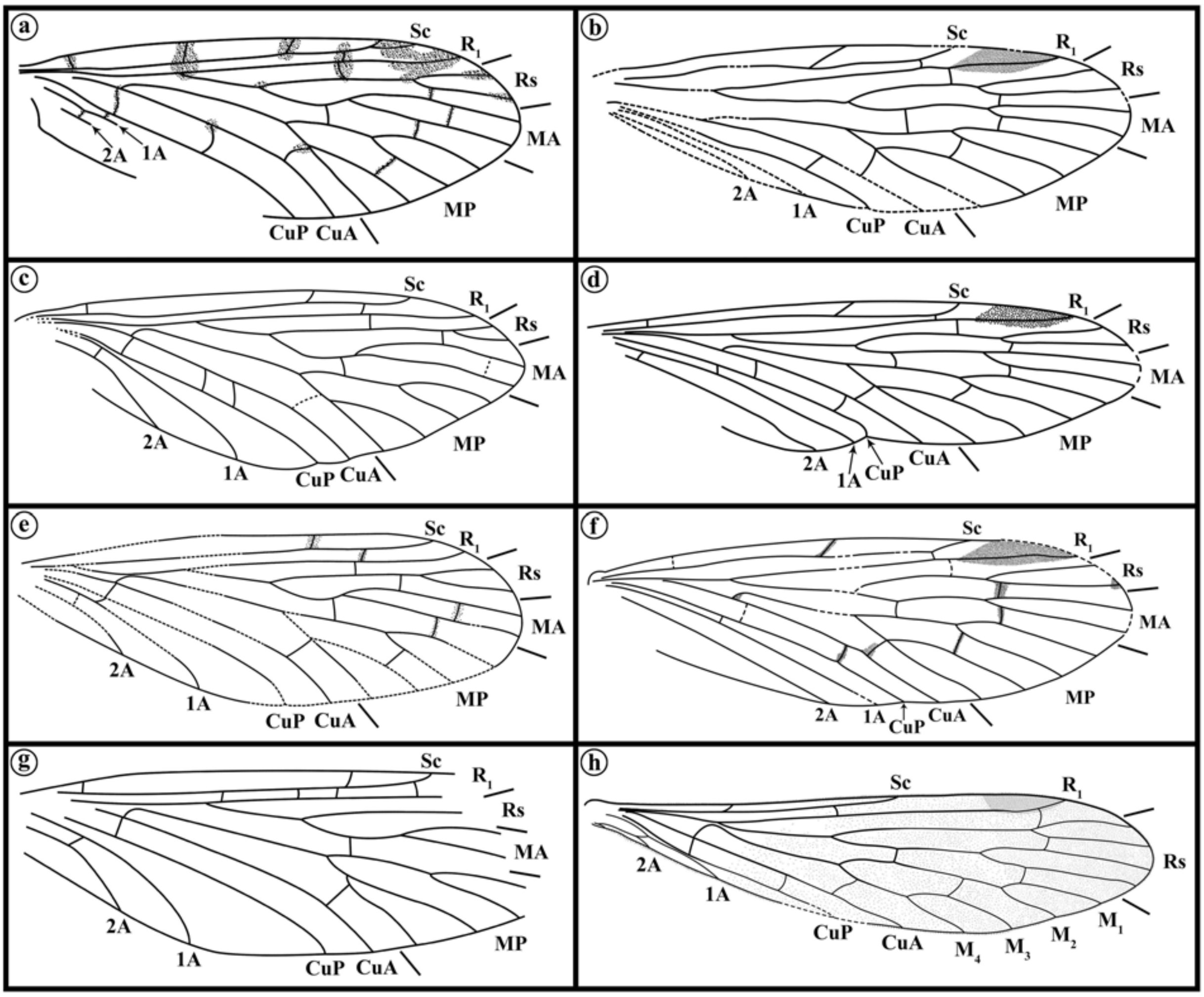
The wing venation of the Dualulidae is similar to that of Mesopsychidae, particularly *Vitimopsyche*[56] and *Lichnomesopsyche*[57]. Although a distinct system of vein nomenclature historically has been used to describe mesopsychid venation, the six principal venational similarities of the Dualulidae and Mesopsychidae are the following. First, the Sc vein of most species has only one anterior branch, excluding *Vitimopsyche torta*[56]. Second, the Rs and MA veins have two branches, and the MP vein has four branches. Third, the CuA and CuP single, unbranched veins are associated with the two anal veins. Fourth, the stem of the MP vein distinctly curves at the base of the wing and forms a near right angle, a condition occurring in *Dualula* and most species of *Vitimopsyche*. Fifth, the origin of the MP vein from the CuA vein is at the same level lengthwise in the wing as the first cua-cup crossvein, seen in *Dualula* and *Vitimopsyche*. Sixth, the origin of the MP vein from the CuA vein is almost at the same level as the origin of the Rs+MA vein from the $R_1$ vein, a feature found in *Dualula* and *Lichnomesopsyche*.

By contrast, the Dualulidae differs from *Vitimopsyche* and *Lichnomesopsy*che in the following six ways. First, the anal field of the wing is distinctly narrow in Dualulidae, compared to a much broader field found in Mesopsychidae. Second, the Sc is relatively short, and much closer to the $MP_{1+2}$ bifurcation in both families than in Dualulidae. Third, the posterior margin of the wing at the CuP vein apex lacks an embayment in Dualulidae and in two species of *Vitimopsyche* (Supplementary Fig. 6a,g). Fourth, the Rs bifurcation is considerably distal to the MA vein and the stem of MA and $MA_1$ does not form an S-shaped vein. Fifth, the $MP_{1+2}$ and $MP_{3+4}$ bifurcations are considerably more distal to the MP vein bifurcation; and the $MP_{1+2}$ much more distal to the $MP_{3+4}$. Sixth, the 1A and 2A are relatively short and contact the wing margin nearly at the same level as the separation of the Rs+MA from the $R_1$ vein (Supplementary Fig. 6.)

The wing and body size of Dualulidae are substantially smaller than most species of Mesopsychidae. We examined three specimens of Dualulidae, two females and one male, and 16 specimens of Mesopsychidae, five female, three male and eight unassigned to sex. The Dualulidae forewing length is 9.4 mm for females and 7.1 mm for males; similarly, their body length is 8.26 mm for females and 7.62

mm for males. Nevertheless, forewing and body length in Mesopsychidae for well-preserved specimens suggest that the size of most Mesopsychidae was small to medium for a mid-Mesozoic insect. For Mesopsychidae, the length of the right forewing ranges from 5.73 mm to 29.02 mm, with an average of 21.35 mm (data includes specimens with one preserved forewing). Except for most species of Permian *Permopsyche*[58] and some species of *Mesopsyche*[41,58,78], the forewing length of all Mesopsychidae species is greater than 21 mm and is 2.23–2.96 times as long as Dualulidae. Moreover, the body size of *Mesopsychidae* is approximately 23.15 mm, 2.46–3.26 times as long as Dualulidae. However, the ratio of forewing length to body length (excluding proboscis and antennae) are minimally different between Mesopsychidae and Dualulidae, ranging from 0.96 to 1.48 and 0.93 to 1.14 respectively.

These data indicate that whereas wing and body size differed considerably between Mesopsychidae and Dualulidae, body shape, or aspect ratios, remained relatively stable. (Supplementary Data 4 and 5.) *Dualula* is very similar to *Parapolycentropus* except for six significant morphological differences. First, unlike *Parapolycentropus*, tergum VIII of males is present in *Dualula*, but is fused with tergum IX or lost in



**Supplementary Figure 6 │ Comparison of forewing venation in *Vitimopsyche*[56], *Lichnomesopsyche*[57] and *Dualula*.** (**a**), Line drawing of *Vitimopsyche torta*[56]. (**b**), Line drawing of right forewing of *Lichnomesopsyche prochorista*[22]. (**c**), Line drawing of *Vitimopsyche kozlovi*[57]. (**d**), Line drawing of *Lichnomesopsyche gloriae*[57]. (**e**), Line drawing of *Vitimopsyche pristina*[22]. (**f**), Left forewing line drawing of *Lichnomesopsyche daohugouensis*[22] (CNU-MEC-MA-2015011P/C). (**g**), Line drawing of *Vitimopsyche pectinella*[59]. (**h**), Right forewing line drawing of *Dualula kachinensis* gen. et sp. nov. (Paratype, CNU-MEC-MA-2017016, a female). Except (h), all others are redrawings based on the published line drawings. Drawings are not to scale.

*Parapolycentropus*. Second, the microstructure of the male genitalia is different in several respects. The gonostylus of *Dualula* is poorly developed, but upturned in *Parapolycentropus*. The gonocoxa dististylus is inflated in its central part in *Dualula*, in contrast to the tapering condition in *Parapolycentropus*. The gonocoxa basistylus in *Dualula* is olive shaped and smooth, in contrast to a V-shaped structure housing a spine in *Parapolycentropus*. Although the shape of tergum IX is distinct in these two groups, the distal tergal edge consists of three or four obvious, cusp-shaped protuberances in *Dualula*, but is composed of a row of denticles in *Parapolycentropus*. In *Dualula* the penis and subsidiary structures are present, while conspicuously absent or invisible in *Parapolycentropus*. Third, the antenna of *Dualula* consists of 20 flagellomeres, of which flagellomeres 1–7 are nearly trapezoidal in shape, whereas in *Parapolycentropus*, only 16 flagellomeres are present and the basal five flagellomeres appear tapered. Fourth, there are several proboscis-related differences between these two taxa. The proboscis is considerably more narrow and slender in *Dualula* than most *Parapolycentropus*, which have a comparatively more robust proboscis. The ratio of length to the width of the proboscis is 32.3 in female and 16.54 in male *Dualula*, in contrast to an average of 14.03 in *Parapolycentropus* regardless of gender. Moreover, the third segment of the maxillary palp is not obviously different in length between male and female in *Parapolycentropus*. However, it is considerably extended in *Dualula* males and less so among *Dualula* females. Notably, a distinctive hypopharyngeal valve occurs in *Dualula*, a condition evidently missing in *Parapolycentropus*. Fifth, in *Dualula* each leg bears two tarsal claws terminally and a thick and long seta in the middle; in distinction, the tarsal claws of *Parapolycentropus* are highly variable, wherein even one individual can express differences in claw form across its prothoracic, mesothoracic and metathoracic legs. In *Dualula*, the tibiae of the metathoracic legs lack sexually dimorphic, robust setae; in *Parapolycentropus*, there is a row of nine thick and sharp setae on the tibiae. Sixth, there are several important differences in forewing venation between these two taxa. In *Dualula*, the Sc vein is about two times as long as it is in *Parapolycentropus*; in similar fashion, in *Dualula* the Sc vein displays one, short anterior branch in contrast to the absence of branching in *Parapolycentropus*. *Dualula* lacks a crossvein between the $R_1$ and C veins, and in *Parapolycentropus,* it is replaced by a sc–r1 crossvein. In *Dualula*, an R vein ends at the C vein in a position considerably proximal to the $Rs_{1+2}$; by contrast, in *Parapolycentropus* this vein ends at the same level or is only in a slightly proximal position. The M vein in *Dualula* has four, long branches from an unmerged vein stem, whereas in *Parapolycentropus* the stem of the M vein has a curved course and forms an almost right angle. *Dualula* lacks a dc cell, which is present in *Parapolycentropus*. The anal field is relatively broad in *Dualula*, with at least two (or three) branches of the anal vein, in contrast to a narrow anal field in *Parapolycentropus* and only one or two branches of the anal vein. The CuP and anal veins are longer in *Dualula* than in *Parapolycentropus*. In *Dualula*, the CuP vein occurs at the same level as the bifurcation of the M; by contrast, in *Parapolycentropus* the CuP vein is present at a position much more distal to the M vein bifurcation. As well, the wing shape varies between the two genera. The ratio of wing length to width is 3.83 in *Dualula* and about 3.0 in *Parapolycentropus*. While there are basic differences between *Dualula* and *Parapolycentropus*, other commonalities in characters and life habits may warrant future placement of *Parapolycentropus* in the Dualulidae or at least as a sister-group to this family.

Many of these morphological differences in wing characters are under the control of homeotic (*Hox*) genes. It long has been known that such genes determine the fate of the fly's second and third thoracic segments, and these genes are particularly involved in wing development[60,61]. The Hox gene *Ultrabithorax* (*Ubx*), is segment specific, avoids the forewing as a target, and expresses wing disc cells of the third segment of the larva, from which the hind wing normally develops. When *Ubx* is expressed at elevated levels in the third thoracic segment throughout larval development, it causes haltere formation in the adult[62]. In this ensuing cascade, the *apterous* gene also targets the *serrate* gene, which provides a ligand attachment to the receptor protein Notch. Notch produces the signaling molecule Wingless[63,64] that in the haltere activates only the anterior compartment, essentially deleting the posterior compartment, resulting in a much abbreviated, elongate hind wing missing its posterior half[65]. Just in front of the anterior-posterior boundary, now essentially forming the posterior boundary of the hind wing, the production of another signaling molecule, Decapentaplegic (Dpp), activates a growth factor TGFß in conjunction with the presence of a second signaling factor, Hedgehog[66,67]. A target activated by the Hedgehog signal is *spalt*, a gene that typically is repressed by the *Ubx* protein, which with Dpp regulates haltere size[68]. Although the hind wings are very similar in *Parapolycentropus* and *Dualula*, *Ubx* and homologous Hox genes control expression of many of the above-mentioned morphological features.

## Future Studies of Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae

Previously, *Parapolycentropus* was a genus along with the other three genera that constituted Pseudopolycentropodidae (Figs. 5,6; Supplementary Figs. 8,9). In the resulting trees (Fig. 1) *Parapolycentropus*, however, is differentiated from these three genera of Pseudopolycentropodidae by body and wing structural characters examined in the phylogenetic analysis. Because of this new placement, it is suggested that additional studies of the systematics of core Pseudopolycentropodidae, *Parapolycentropus* and *Dualula* be made, with the goal of revising the classification of *Parapolycentropus*. It appears that three potential results for placement of *Parapolycentropus* are possible. First, *Parapolycentropus* is closely related to *Dualula* and it may be included as the second genus within the Dualulidae. Secondly, *Parapolycentropus* may belong to a new lineage, perhaps with a sister-group relationship to Dualulidae, where currently it is placed in the tree. Third, and more remotely, it may be reintegrated with core Pseudopolycentropodidae, perhaps as a sister group to the *Pseudopolycentropodes* + (*Pseudopolycentropus* + *Sinopolycentropus*) clade[35,49,77]. Several features contrast *Parapolycentropus* to other Mecoptera taxa, the most significant are wing features, particularly severe hind-wing reduction and features of forewing venation and shape[50] (Figs. 5; 6a,b; 7a,b; Supplementary Figs. 9a,b; 15). *Parapolycentropus* also shows considerable similarities to *Dualula*, particularly in mouthparts (cibarial and salivary pumps) and hind wing reduction (Figs. 1a,b,d,e; 2; 3; 6a,b; 7; Supplementary Figs. 2–5; 8; 9).

The phylogenetic analysis also revealed a close relationship between (*Parapolycentropus* + Dualulidae) + (Siphonaptera + basal Diptera) with Aneuretopsychina *sensu lato* in the next subjacent node. The Aneuretopsychina clade consists of Pseudopolycentropodidae + (Liassophilidae + {Permotanyderidae + [Aneuretopsychidae + ‖Mesopsychidae + Nedubroviidae‖]}), the major lineages of which have highly variable times of origin. Of the four demonstrable long-proboscid clades, Mesopsychidae has a Late Permian time of origin in Australia[26] and a slightly younger occurrence from the Permian–Triassic boundary interval of Russia[58,78]. Nedubroviidae has a latest Permian time of origin[79] and an Early Triassic occurrence[40,79]. Pseudopolycentropodidae has an early Middle Triassic occurrence from France[76]; and earliest Aneuretopsychidae is from the Late Jurassic of Kazakhstan[85]. For the two possible long-proboscid clades, earliest Permotanyderidae occurs in the Late Permian of Australia[26]; and Liassophilidae has an earliest occurrence during the Early to Middle Triassic of France[81,124,125]. Although this disparity in the times of origin is attributable to a poor fossil record of terrestrial deposits, it provides evidence that Aneuretopsychina extends deep to the late Permian (Lopingian), had a first phase of early lineage diversification during the Early to Middle Triassic, and a subsequent phase of later lineage diversification in the Middle Jurassic to Early Cretaceous. Based on the trees in Figure 4, the Diptera would have had an origin during the Late Permian, although the earliest occurrence of a well-documented basal dipteran in Figure 1 is *Gallia*, assigned to Rhagionidae, of Early–Middle Triassic age[81,83].

## Supplementary Note 2 │ Mouthparts of Long-Proboscid Mecoptera

Lineages within the traditional Aneuretopsychina – the assemblage or possibly clade that includes Mesopsychidae, Aneuretopsychidae, Pseudopolycentropodidae, Nedubroviidae and initially *Parapolycentropus* and the new family, Dualulidae, described in this report – are united by long-proboscid mouthparts[21]. These mouthparts display significant, important differences in structural details among these lineages. In Mesopsychidae, the proboscis is long, and consists of a closed, tubular siphon with an external surface randomly covered with thick setae[21], but lack the series of encompassing, sclerotized, annular rings reported here in Dualulidae. In addition to the absence of sclerotized bands, the mesopsychid proboscis lacks external ornamentation such as transverse ridges that define the Aneuretopsychidae[21]. Pseudopolycentropodidae by contrast contains diminutive sclerotized bands with microtrichia, which often give the appearance a smooth, featureless surface in compression fossils[49]. Ovoidal labial pads, the pseudolabellae, occur prominently at each side of the proboscis terminus in Mesopsychidae, are structurally quite different in Aneuretopsychidae, and absent entirely in Pseudopolycentropodidae[21,49,50] and Dualulidae. Individual mouthpart elements of the Mesopsychidae proboscis of one specimen were observed as separated into two, perhaps three, elongate structures that probably represent tongue-and-groove interlocking features[21] that keep the galeal walls of the proboscis intact and tubular. By contrast, the most distinguishing proboscis characteristic of Aneuretopsychidae is the distinctive, transverse, annular ridges and dense setae or microtrichia arranged perpendicularly along these ridges on the external proboscis surface[21,39,83]. Aneuretopsychid pseudolabellae consist of a distinctive, large, U-shaped pseudolabellum that probably increases the contact area with the substrate, allowing for more conducive absorption of surface fluids. Other elements of the mouthpart complexes of Aneuretopsychina are poorly known. However, the mesopsychid maxillary palp has three robust articles, apparently is positioned abutting along the labrum, and has a third segment curved in shape and slightly shorter than other two. Aneuretopsychidae evidently houses a cibarial pump below the clypeus[21,83] that provides for the flow of incoming food along the proboscis.

The directional deployment of the Aneuretopsychina proboscis is important for understanding the life habits of these lineages. For Aneuretopsychidae the proboscis was deployed in a backwardly directed, opisthognathous position. For Pseudopolycentropodidae and Dualulidae, the proboscis was directed in a forwardly jutting prognathous position, as in Mesopsychidae[21,49,50]. These two proboscis orientations are similar to mouthpart placement in sternorrhynchan Hemiptera and Lepidoptera, and contrast with the mostly hypognathous position of mouthparts in extant Mecoptera[21,84]. For Nedubroviidae, because of poor preservation of the proboscis, the most recognizable feature is the base of the proboscis, which includes a triangular and enlarged labrum, possibly partly fused with the clypeus that supports an extended rostral base and a robust, prognathous proboscis[79]. These proboscis orientations indicate that their proboscides were used for different purposes and can be contrasted to other, contemporaneous, long-proboscid lineages. Various lineages of Aneuretopsychina had particular directional feeding contingent on orientation of ovulate organs for access. For example, the tips of small *Williamsonia* organs likely were oriented in an upward manner whereas *Caytonia* integumental tubules were directed downwardly[21,84], allowing for different positions of the head and extended proboscis for efficient access to pollination drops.

Of the several, long-proboscid lineages of the Aneuretopsychina, Pseudopolycentropodidae is one of the more closely related lineages to Dualulidae. Various body features of Pseudopolycentropodidae, particularly mouthparts, inform interpretation of Dualulidae mouthpart structure and function, as both lineages exhibit similar structures. Pseudopolycentropodidae (Supplementary Fig. 7) and *Parapolycentropus* (Figs. 5a,b; 6; 7; Supplementary Figs. 8; 9a,b), have a proboscis that is formed from three separate, basic parts – two galeal halves that are joined to form a tube, and an elongate hypopharynx that evidently is variously modified to serve different mouthpart-related functions. Labral and labial elements are well developed, providing bracing to the proboscis; mandibles are absent; and the maxillary region is often reduced. The proboscides in some taxa have dense, annular microtrichia and sclerotized bands on the outside surface of the galea; a maxillary palpus consisting of three articles, the third one is slightly longer than other two; and the absence of terminal absorptive structures such as pseudolabellae[49,50,69,77].

Five major comparisons exist for the mouthparts of Mesopsychidae, Aneuretopsychidae, Pseudopolycentropodidae, Dualulidae and *Parapolycentropus*, the latter formerly a member of the more phylogenetically distant Pseudopolycentropodidae but now closely related to the Dualulidae probably as a sister group (Fig. 1). First, Dualulidae, *Parapolycentropus* and Aneuretopsychidae have a distinctive external ornamentation and setal insertion patterns on their proboscides[39,49,50,69,83]. Mesopsychidae possessed randomly distributed, thick microtrichia and Pseudopolycentropodidae much less so, lacking recognizable surface structures as compression fossils[21,69]. Second, Aneuretopsychidae have a highly specialized and large, apparently fleshy, pseudolabellum that likely was a single structure[39,83]; by contrast, Mesopsychidae bore more diminutive pseudolabellae that were separated as two fleshy lobes on opposite sides of the proboscis terminus[21]. Pseudolabellae evidently do not occur among other mid-Mesozoic long-proboscid insects, although the proboscis in much older Permithonidae apparently had a modified (albeit incomplete) proboscis terminus with sclerotization and projecting setae[84]. Third, except in Aneuretopsychidae and Nedubroviidae that lack evidence for a hypopharynx, other long-proboscid groups clearly can be observed to have three parts – two conjoined galeal elements to form a tube and an intra-tubular hypopharynx. Additionally, Dualulidae and Pseudopolycentropodidae lacked the tight interlocking mechanism joining the galeal halves proposed for Mesopsychidae[21]. Most compression fossils of Pseudopolycentropodidae are preserved as sutured mouthpart elements evident along a section of the proboscis or are entirely disarticulated from the proboscis base (Supplementary Fig. 7). Fourth, although all long-proboscid taxa house a cibarial food pump under the clypeus, as well as an accompanying food tube, the much smaller salivary pump with a salivarium and salivary duct has only been securely established in Dualulidae (Supplementary Figs. 3a–e; 5b) and *Parapolycentropus* (Fig. 7d; Supplementary Figs. 8b–h; 9c–h). For functional reasons it is likely that Mesopsychidae and Aneuretopsychidae[20] had a salivary pump, but confirmation would require better preservation of additional specimens than exists at present[21,39,57,58,83,85]. Fifth, there is considerable variety in mouthpart movement and flexibility across the lineages. Some taxa can rotate or twist their proboscides almost 360°, such as Dualulidae and *Parapolycentropus*. Other taxa are significantly more limited in proboscis torsion and bending. Some had an ability to slightly bend their proboscides in a fixed direction, such as Mesopsychidae and most Pseudopolycentropodidae, whereas others taxa possessed a relatively stiff proboscis that disallowed any significant flexing, principally Aneuretopsychidae.

To more fully explore mouthpart microstructure, we took SEM scanning images of three specimens of *Pseudopolycentropus janeannae*[21], focusing on the base, midsection and terminus of each proboscis (Supplementary Fig. 7). At the base of the *P. janeannae* proboscis, the hypopharynx is largely covered by conjoined galeae; the hypopharyngeal salivary duct is inconspicuous. The maxillary palp is short, adpressed to the side of the labrum. At mid-proboscis, the hypopharynx is separated from the inner proboscis (galeal) surface, and it is at this point that sclerotized bands are clearly observed on the outer surface of the proboscis. This observation, especially in compression fossils, challenges previous statements that the only external structures on the proboscis were annular setae or microtrichia. At its terminus, the proboscis is split along the galeal suture, with only one galea is visible in a lateral, inner view, revealing the food tube inner surface that is very smooth compared to the uneven outside surface.

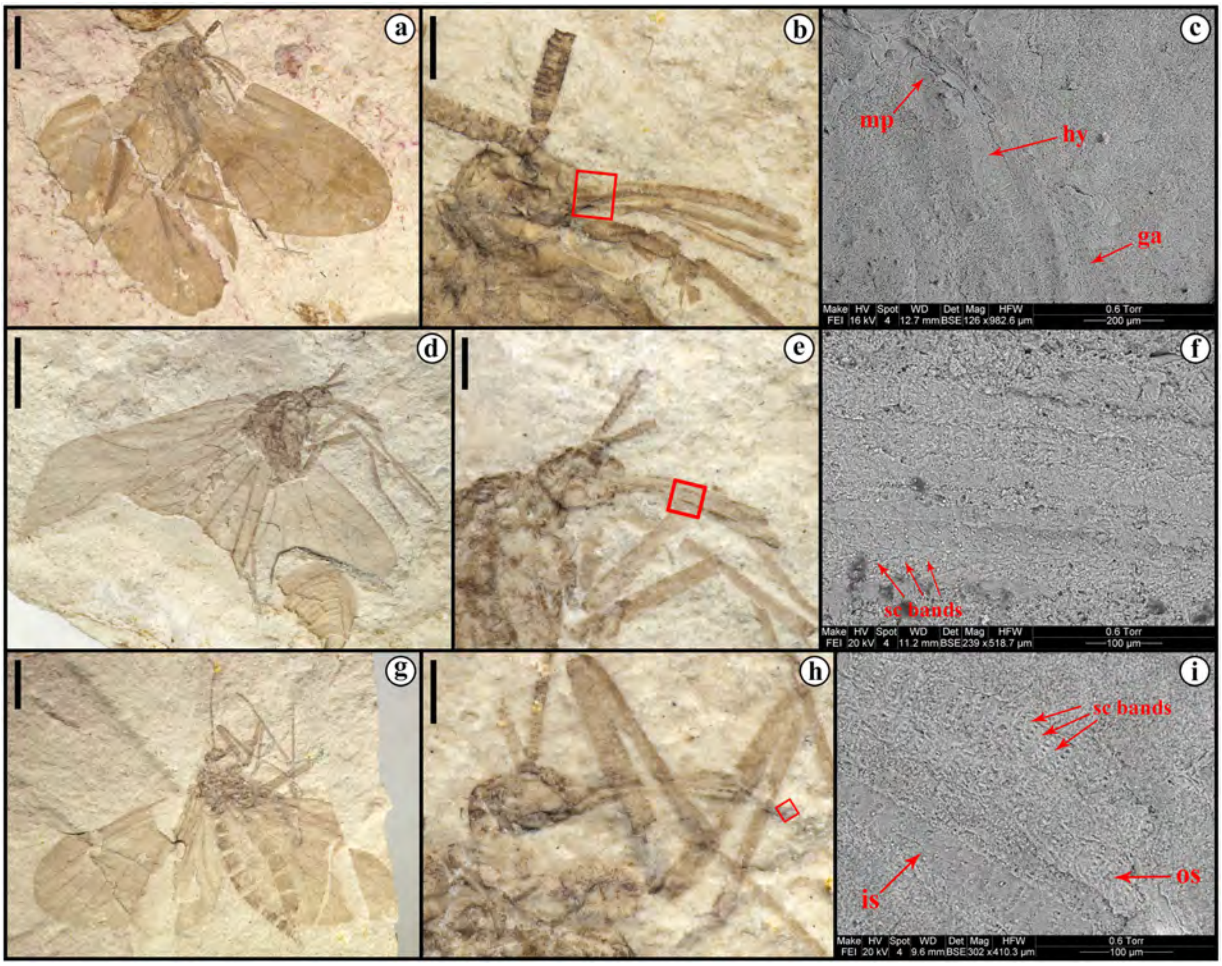
Compared to compression material, amber specimens display a greater number and more detailed features than compression specimens when observed under a stereoscopic microscope. For example, most specimens of amber *Parapolycentropus* reveal a delicate head and mouthpart microstructure, which can provide additional details when such features are viewed from different lighting angles. One pair of dark, maxillary sclerites clearly can be seen in ventral view, consisting of a small, somewhat angulate cardo ridged in lateral view, which articulates with a longer and slender stipes that in turn connects with the palpifer (Supplementary Figs. 5b; 8a–c). The maxillary palp is three-segmented, occasionally is adjoined to the side of the labrum, but is physically separated from the proboscis (Figs. 3; 6d; 7h–j; Supplementary Fig. 8c–f,h). Although judging from its structure the palp is capable of considerable movement and flexibility, it is far too short for tactile contact of food at the proboscis terminus. However, some of these activities may be provided by an abbreviated maxillary palp with its three articles. The first article is short, thick, nearly a rectangular solid in shape, and principally serves as a connecting and supporting structure with the palpifer and stipes. The second maxillary article is fusiform and laden with several oval sensillae on the lateral surface, each including a macrotrichium in the center. The number and size of the various sensillae on the second maxillary article is uncertain, and may vary across specimens. The third, terminal maxillary article is substantially more slender than other two, and is densely covered with setae and sometimes upwardly oriented. Straddling the paired maxillary cardo and stipes sclerites on each side are the medially placed mentum of the labium, the most posterior of the head segment regions (Fig. 7j; Supplementary Figs. 5a,b; 8a–c). The mentum sclerite lies anatomically in the center of the ventral head region and often joins anteriorly the prementum. The prementum often is well developed, and it distinctly protrudes anteriorly where it adjoins the hypopharynx. The submentum is a triangular sclerite that is positioned posteriorly, is typically reduced in size and, sometimes is an inconspicuous, median sclerite. These labial sclerites – submentum, mentum and prementum – collectively appear elongate-rectangular in shape, are often vestigial, and are much narrower than the cardo and stipes of Pseudopolycentropodidae.

For Pseudopolycentropodidae, *Parapolycentropus* and *Dualula*, the clypeus and labrum have a smooth, external surface and house, respectively, the cibarial and salivary pumps below these sclerites. The cibarial pump, or food pump, provides suction through an expanding and contracting cibarial chamber for imbibition of incoming fluids through the proboscis food tube, and is substantially larger than the salivary pump. The salivary pump contains a chamber, the salivarium, which is attached to a valve (only preserved in *Dualula*) that allows the expelling of outgoing fluids through the narrow salivary duct at the proboscis terminus. These pumps likely are controlled by a series of compressor (cibarial pump) or circumferential muscles (salivary pump) that create negative and positive pressure, respectively, through the expansion and contraction of the cibarium and salivarium chambers visible in dorsal and lateral views (Fig. 3; Supplementary Figs. 8b–h; 9c–h; Supplementary Video 1). Between the inverted triangle of three ocelli above and the two antennal scapes below, a pair of frontal flanges (only observed in *Parapolycentropus*) are present on the head frontal surface (Figs. 6d; 7i; Supplementary Fig. 8b,d). Four or five types of frontal flanges are present in a variety of individuals in lateral view, consisting of columnar, semicircular, low-arched, oblique-triangular and irregularly shaped forms, but without an evident sex-based pattern. These unique structures could be the external expressions or the buttressing of internal apodemes for compressor muscle insertions to the cibarial pump. Connected to the cibarial pump are the long and flexible and conjoined galeae that form the food tube, consisting of an internally smooth surface, but with sclerotized, circumferential bands and annular setae on the external surface (Figs. 3; 6d,e,i; 7f; Supplementary Figs. 5a,b; 7; 8i–k,m,o). The food tube of conjoined galeae contains a hypopharynx that is slender, almost pellucid, and includes serrations on its center-ventral surface. Other than the serrations, the outer hypharyngeal surface is smooth, and lacks and setae, microtrichia, or a terminal acuminate or serrated structure for puncturing. It is notable that the hypopharynx preserves in its terminus, at the opening of the salivary duct, a small bubble with exuding fluids (Supplementary Fig. 8p, left). This indicates that there was outflow of fluids from the salivary duct, encouraged by the trauma of resin entombment. As well, there also are parallel exudations from the food tube resulting in much larger bubbles of egested fluid (Supplementary Figs. 8p–r).

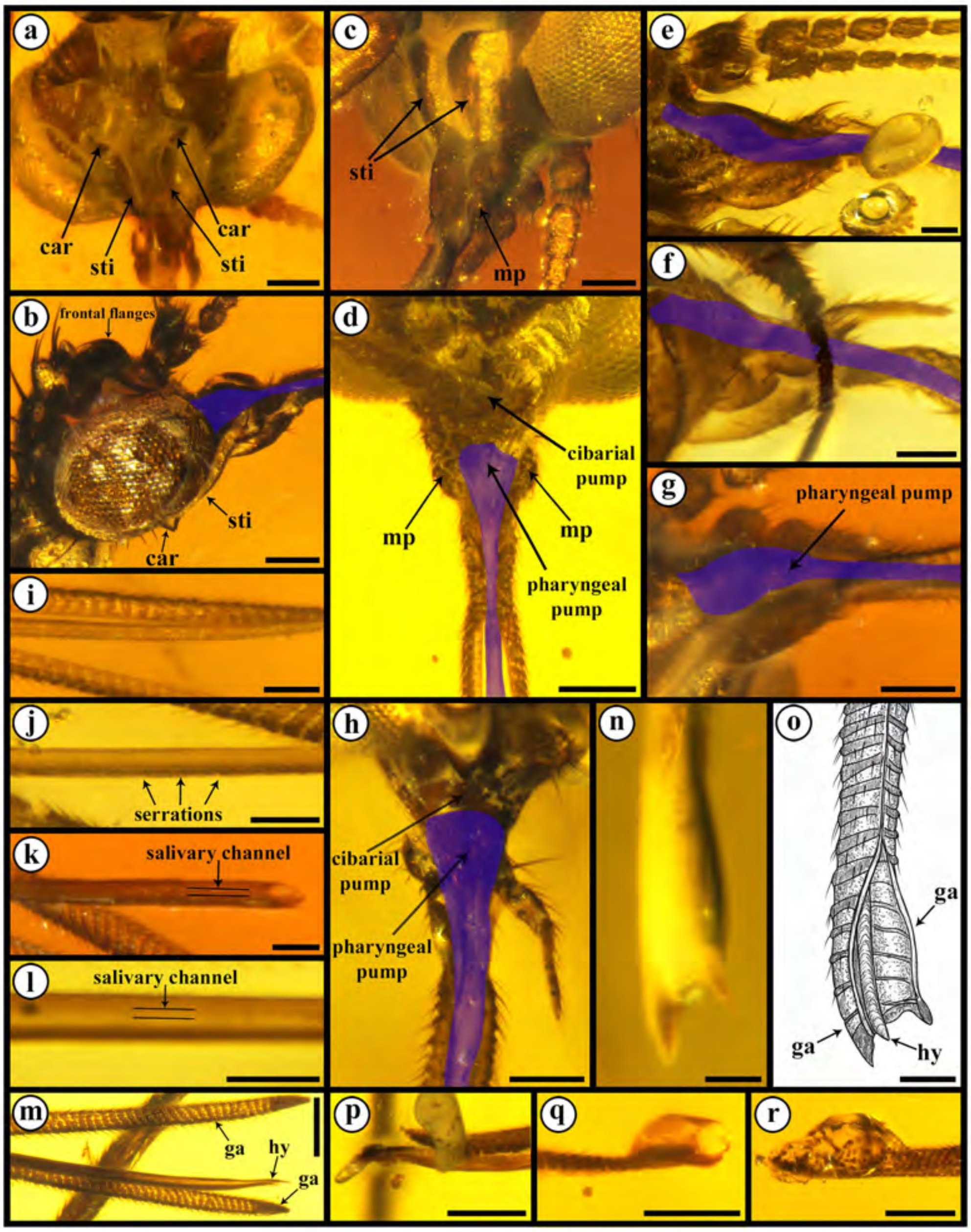
## Supplementary Note 3 │ *Dualula* Mouthpart Structure

The proboscis of *Dualula kachinensis*, monotypic member of Dualulidae (Figs. 1g–i; 3; Supplementary Figs. 3a–e; 5a,b), is a unique structure compared to closely related Pseudopolycentropodidae and *Parapolycentropus*[21,49,50,69,77], other, mid-Mesozoic, long-proboscid Mecoptera[21,49,50,84] (Supplementary Figs. 7; 8a–h; 9c–h), and remaining long-proboscid insect lineages past or present[84,86–90]. The two, major, active elements of the dualulid fluid-feeding apparatus are the modified hypopharynx with its supporting pump, and the surrounding maxillary galeae conjoined into an enveloping food tube that also houses its supporting pump.

The anatomically anteriormost elements, the clypeus and labrum, lack external ornamentation except for short peripheral setae and subtle labral ridges. The domed, polygonally shaped clypeus modified into a salivary pump. The maxillary elements of the head that provide the tubular galeae



**Supplementary Figure 7 │ Proboscis details of *Pseudopolycentropus janeannae***[21,49]. These specimens represent new material. (**a**), Specimen CNU-MEC-NN-2016008, sex unknown. (**b**), Specimen displaying thorax, head and proboscis, from (a). (**c**), SEM image enlarged from the template at proboscis base in (b). (**d**), Specimen CNU-MEC-NN-2016001P; sex unknown. (**e**), Enlargement of (d), showing thorax, head and proboscis. (**f**), SEM image enlarged from the template at mid proboscis in (d). (**g**), Specimen of CNU-MEC-NN-2016015P; female. (**h**), Enlargement of (g), showing thorax, head and proboscis. (**i**), SEM image enlarged from the template at proboscis tip in (h). Abbreviations: **ga**, galea; **hy**, hypopharynx; **is**, inner surface; **mp**, maxillary palp; **os**, outer surface; **sc bands**, sclerotized proboscis bands. Scale bars represent 2 mm in (a), (d) and (g); and 0.5 mm in (b), (e) and (h).

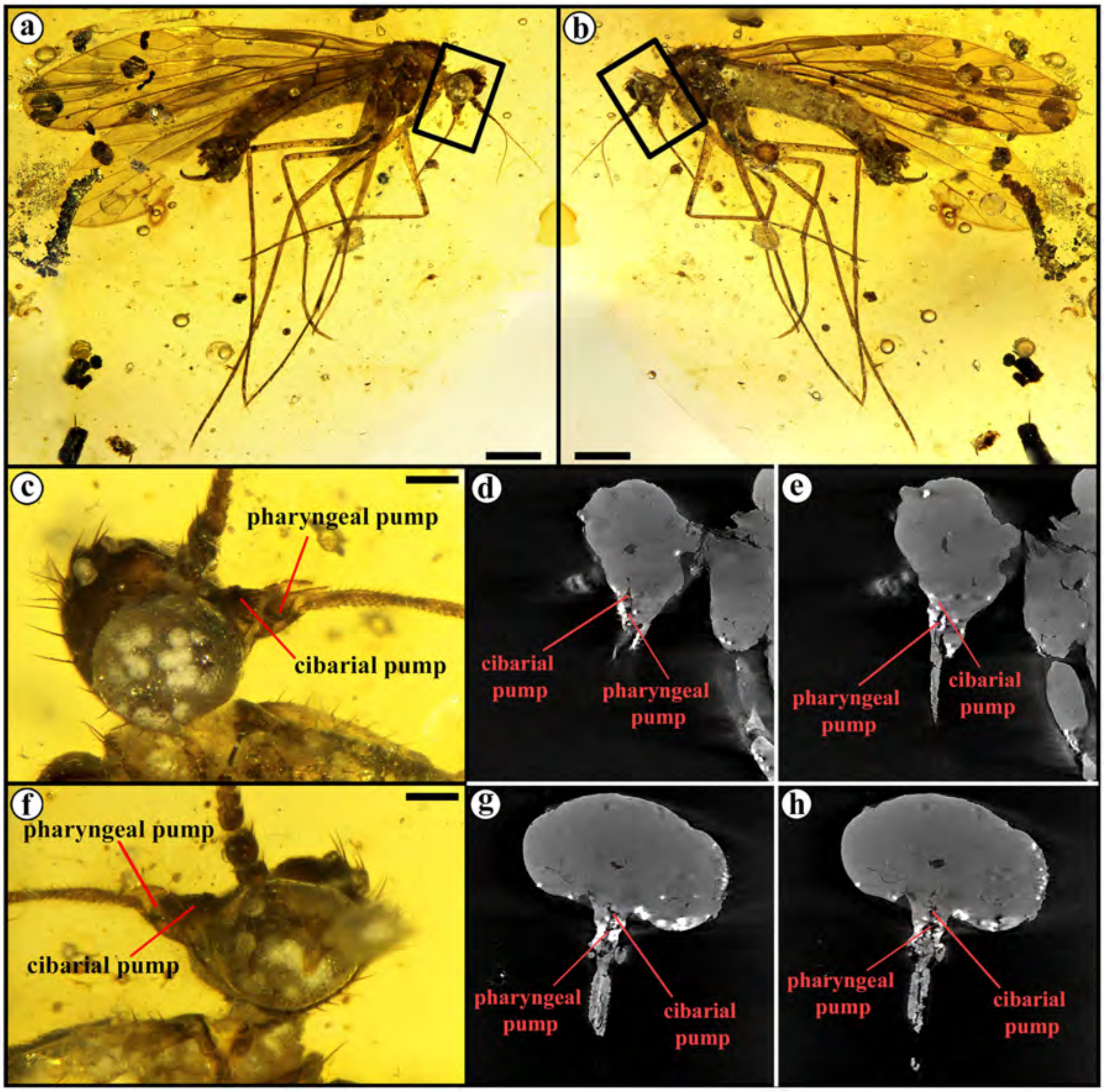


**Supplementary Figure 8 │ Details of the proboscis base and other mouthpart elements of *Parapolycentropus* spp[50].** These specimens are new material. (**a**), Ventral view of head and mouthparts CNU-MEC-MA-2014005. (**b**), Right lateral view of head and mouthparts CNU-MEC-MA-2015052. (**c**), Ventral view of head and mouthparts CNU-MEC-MA-2015048. (**d**), Dorsal view of proboscis base CNU-MEC-MA-2015054. (**e**), Right lateral view of proboscis base CNU-MEC-MA-2017020. (**f**), Right lateral view of proboscis base CNU-MEC-MA-2015036. (**g**), Right lateral view of proboscis base CNU-MEC-MA-2015048. (**h**), Dorsal view of proboscis base CNU-MEC-MA-2014003. (**i**), Right lateral view of mid proboscis CNU-MEC-MA-2015055. (**j**), Left lateral view of hypopharynx CNU-MEC-MA-2017015, exhibiting serrations. (**k**), Right lateral view of hypopharynx CNU-MEC-MA-2015048, indicating the salivary duct. (**l**), Right lateral view of hypopharynx CNU-MEC-MA-2017007, with the salivary duct demarcated. (**m**), Right lateral view of proboscis terminus CNU-MEC-MA-2016016. (**n**), Dorsal view of proboscis terminus CNU-MEC-MA-2015037 in dorsal view, with its superimposed line drawing at (o). Lateral views of the proboscis termini CNU-MEC-MA-2017009 in (**p**), CNU-MEC-MA-2017013 in (**q**) and CNU-MEC-MA-2017014 in (**r**), showing extrusion of proboscis fluid during the resin entombing process. Purple denotes hypopharyngeal elements, including the basal, expanded, salivarium pump and the thin salivary duct to the terminus. Abbreviations: **car**, cardo; **ga**, galea; **hy**, hypopharynx; **mp**, maxillary palp; **sti**, stipes. Scale bars represent 0.1 mm in (a)–(d), (g), (h), (m) and (p)–(r); and 0.05 mm in (e), (f), (i)–(l), (n) and (o).

surrounding the salivary-pump complex originate from sclerites in the ventral part of the head capsule. These maxillary sclerites link to the proboscis base and occur lateral to the medially placed and often inconspicuous, elongate labial sclerite, the mentum. Each laterally placed set of maxillary sclerites consist of two elements: a small, angulate shaped cardo near the base of the head capsule and an articulating, relatively slender stipes that connects with the proboscis base and other maxillary elements (Supplementary Fig. 5a,b). The distal aspect of each stipes bears a lateral bulbous appendage, the palpifer, which supports the maxillary palpus, each composed of three compact articles and a terminal, filiform article that extends about two-thirds of the distance to the proboscis terminus. In some specimens, sensillae dot the surface of the second article. Another sex-based difference is the length of the third article in male Dualulidae (Supplementary Figs. 3a–d; 5a,b), which is about one-third of the length as the second article; by contrast, in females the same article is slightly longer than the second article. The highly reduced labium lacks palpi or palpifers, and occupies the narrow interstice between each lateral, articulating cardo–stipes pair. In some specimens, a triangular prementum sclerite is present and occasionally displays subtle features such as ridges.

The first and larger pump is the cibarial pump[91] that occurs under the clypeus and found in virtually all insect lineages regardless of mouthpart type. The cibarial pump in Dualulidae is evidenced by a bulge of the clypeus (Fig. 1i; Supplementary Fig. 5a,b), where it is supported on the surface by the clypeal sclerite (Fig. 3i; Supplementary Fig. 3d). The cibarial pump is powered by compressor musculature attached to an expandable chamber, the cibarium, for creation of suction via the esophagus for imbibition of fluid from the proboscis food tube[92]. In Dualulidae, the food tube of two sutured galeae is prominently ornamented by a series of sclerotized rings along each galeal half of the tube, and is diminished in intensity near the proboscis base and terminus. However, an alternative interpretation of the function of the conjoined galeal siphon recently has been proposed[69]. This earlier view, a misinterpretation in our view, posits the galeal siphon in Pseudopolycentropodidae as not a food tube, and rather serves a protective role by encompassing the salivary duct. Accordingly, the salivary duct was the proposed conduit for imbibition of fluid food[69]. Nevertheless, the salivary duct, because of its considerably narrower diameter than the food tube, would encounter resistance from inertial forces[93] that result from consumption of viscous food, disallowing passage of fluids that typically would flow freely in the galeal siphon of much wider diameter. Enzyme rich salivary fluids are considerably less viscous[94] and could be extruded through a salivary duct of much narrower diameter.

The second and smaller pump under the labrum is the salivary pump[91], a much smaller structure than the cibarial pump and is present in almost all piercing-and-sucking insects. The salivary pump in Dualulidae is a modification of the hypopharynx that consists of a hypopharyngeal base repurposed as a chamber, the salivarium, which serves as a contractile pump within the base of the galeal siphon. In *Parapolycentropus*, the well-exposed contractile salivarium (Supplementary Figs. 8b–h; 9c–h; Supplementary Video 1) contains salivary fluids whose outflow is regulated by a distally placed valve that is opened or closed with two, opposing dentate projections, as seen in *Dualula* (Fig. 3; Supplementary Fig. 3a–d). This condition is different from a valve with a single projection in extant aphids[95]. The salivarium valve controls outflow of salivary fluids along the tubular salivary duct of narrow diameter that extends to the distal proboscis terminus (Figs.1g,h; 3; Supplementary Figs. 3e; 5a,b), where it contacts fluid food on plant surfaces. This long salivary duct has a smooth outer surface, but displays regularly spaced serrations on along its ventral side, possibly serving as an attachment to the food tube inner surface. Notably, a salivarium valve was not detected in closely related *Parapolycentropus* or other Pseudopolycentropodidae, nor reported in other mid-Mesozoic scorpionflies. This absence, especially for compression specimens, may be attributable to insufficient preservation that would reveal microstructural features of the salivarium. Consequently, it is uncertain if other long-proboscid scorpionfly clades possessed a salivary pump



**Supplementary Figure 9 │ Proboscis of *Parapolycentropus paraburmiticus*[50].** This figure includes Micro-CT images indicating cibarial (food) and salivarium (hypopharyngeal) mouthpart pumps from CNU-MEC-MA-2017008 (new material, male). (**a**)**,** Insect in right lateral view. (**b**), Insect in left lateral view. (**c**), Details of head and proboscis base in right lateral view. (**d**) and (**e**), Micro-CT image of the head and proboscis base in left lateral view, indicating the cibarial and salivary pumps in two sections. (**f**), Details of head and proboscis base in left lateral view. (**g**) and (**h**), The head and proboscis base in dorsal view, indicating the cibarial and salivary pumps in two sections. Scale bars represent 0.5 mm in (a) and (b), and 0.1 mm in (c) and (f). Images (d)–(h) are not to scale. For results of Micro-CT scanning, also see Supplementary Movie 1.

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## Supplementary Note 4 │ Feeding Processes and Food Sources of *Parapolycentropus* and Dualulidae

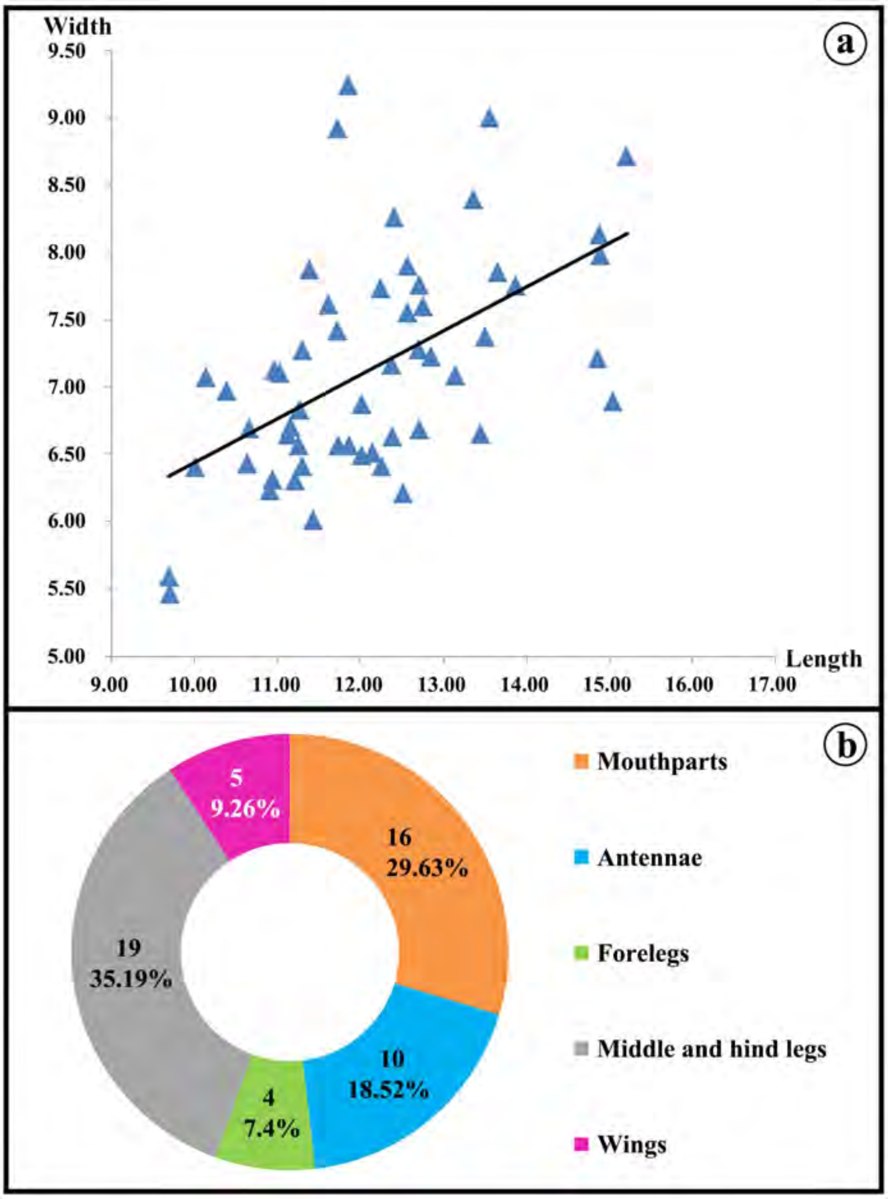
Mid Mesozoic, long-proboscid scorpionflies have been documented as probable pollinators of several extinct gymnosperm lineages and possibly early angiosperms. The evidence for biotic pollination has been overwhelmingly indirect, consisting of mouthpart structure[21,39,77,83] and features of microsporangiate organs, especially ovulate organs[21,94,96], consistent with access to rewards such as pollination drops, nectar and pollen[94]. Four major insect lineages of mid Mesozoic insects bear undisputed pollen grains and clumps adjacent insect bodies: Thysanoptera[97], Coleoptera[98,99], Neuroptera[86] and Diptera[89,96].

These lineages possessed highly specialized mouthpart structures and feeding habits[86,98], and were covered with pollen from one of the four groups of gymnosperms: Cycadales[99], Bennettitales[88], Pinales[86,96] and Ginkgoales[97]. Innovations such as small size, highly maneuverable wings, long-proboscate mouthparts and specialized feeding mechanisms enabled use of food sources associated with pollination[84,94]. A variety of evidence – mouthpart morphology, ovulate organ structure and insect associated pollen – are collectively important in understanding insect feeding habits, pollination and their co-associational relationships with plants.

## Associations with Gymnosperms

The pollen grains mostly were distributed on the surface or adjacent the mouthparts and the middle and hind legs; fewer grains occurred next to the antennae; and the least number of grains were associated with the wings and forelegs (Fig. 6; Supplementary Fig. 10; Supplementary Data 3). For the same 54 pollen grains assessed, the average dimension is 12.15 μm for the long axis (range 9.69–15.21 μm) by 7.17 μm in equatorial diameter (range 5.46–9.24 μm), with a length to width ratio of about 1.70 (range 1.28–2.18) (Fig. 6c; Supplementary Data 3). The sulcus is elongate and almost the same length as the total length of the grain, and is slightly narrower in the middle, where the grain wall enrolls inwardly. The exine is opaque and psilate in ornamentation. The *Cycadopites* grains discussed here (Fig. 6) are substantially smaller than similar grains found on other Mesozoic insect bodies, such as five to eleven million-year-older Early Cretaceous Álava amber of Spain. Approximately 150 Álava amber *Cycadopites* grains of probable ginkgoalean affinity were described attached to and adjacent the bodies of *Gymnopollisthrips*, a melanthripid thrips[97]. These attached and associated grains had average dimensions of 20.4 μm in length (range 17.4–24.9 μm) by 12.6 in equatorial diameter (range 9.3–15.4 μm)[97]. By contrast, the probable cycad affiliated *Monosulcites* pollen on the oedemerid beetle *Darwinylus* had average dimensions of 25.14 μm in length (range 38.57–18.85 μm, N=62) by 16.56 μm in equatorial diameter (range 11.22–28.11 μm, N=69)[98]. This pollen type on *Darwinylus* possessed a rather open, granular sulcus suggesting cycad affinity. The relevant affiliation accommodated by the 12.15 μm average length of our *Cycadopites* sp. pollen grains (Fig. 6; Supplementary Fig. 10; Supplementary Data 3) remains unknown, although some bennettitalean pollen grains approach these small pollen grains in size[100]. Very few described *Cycadopites* taxa approach this size; the closest is a dispersed pollen of unknown affinities from the Late Cretaceous of Austria with a diameter about 12–15 μm in long axis[101]. Notably, *Cycadopites* pollen has not been found to occur in any angiosperm flower. In addition, small, bowl-shaped, neutral hued, nonshowy angiosperm flowers like *Amborella*[96,99] antedate by a few tens of millions of years[98] the earliest occurrence of tubular, deep-throated flowers consistent with long-proboscid pollination[102,103].

Insect pollinators of *Tropidogyne* and other cup-shaped flowers, based on their floral structure and evidence from pollination modes of modern basal angiosperms[96,103], would have been small beetles, midges, small nematocerous flies, thrips, parasitoid wasps and early moths[96,102,103]. Large-bodied insects with considerably larger long-proboscides (Supplementary Data 4) could not interact for a lack of fit. Pollinators with appropriate proboscides would include small, mosquito-sized pseudopolycentropodid, *Parapolycentropus* and dualulid scorpionflies that functionally would have been similar to early moth pollinators. Notably, angiosperm nectar would have been an uncommon resource given the rarity of floral nectaries on basal angiosperm lineages[99,102], and may have replaced pollination-drop consumption as a reward in many insect groups[94]. Although the two *Parapolycentropus* specimens described here are associated with two different types of pollen grains, gymnosperm and, possibly, angiosperm, these disparate associations may record the transition from a gymnosperm to angiosperm plant hosts during the Aptian–Albian Gap[98,103]. Such a transition would be similar to that of Oedemeridae, a lineage of beetles that included Early Cretaceous cycad or bennettitalean as well as modern angiosperm pollinators[98]. The difference, however, between the two examples is that Oedemeridae successfully



**Supplementary Figure 10 │Measurements and distribution of *Cycadopites* sp. pollen grains adjacent to *Parapolycentropus paraburmiticus*.** CNU-MEC-MA2017012, new material, male. (**a**), Scatter plots of pollen grain length and width, and there is a trend line to denote the changes in pollen size. Horizontal axis is the length of pollen, and vertical axis is width, measurements in micrometer (μm). (**b**), Doughnut chart shows the distribution of pollen grains associated with the insect body, the numbers on different color blocks are the specific number (above) and percentage (below) of the pollen grains.

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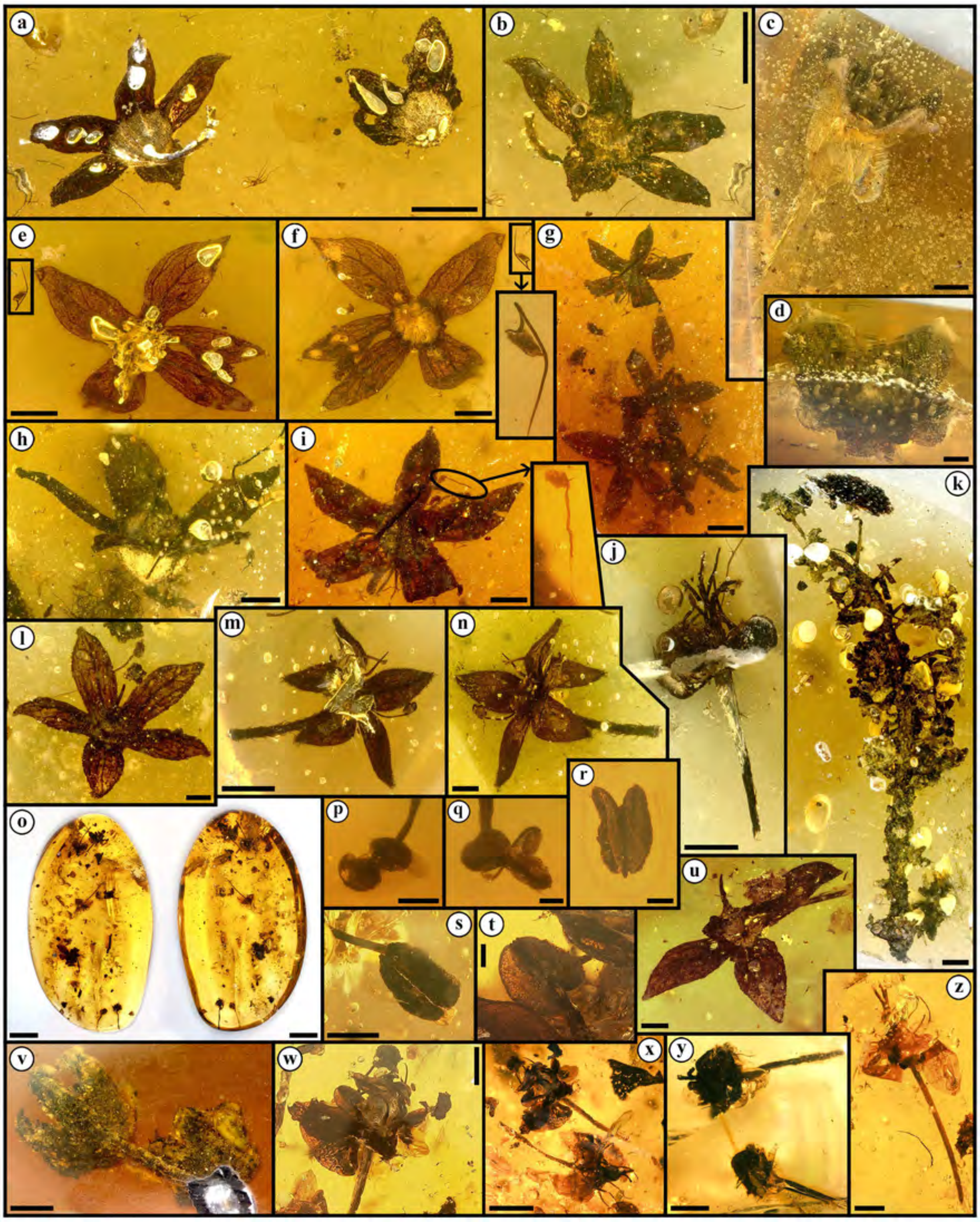
transitioned and is extant, whereas the fossil record indicates that *Parapolycentropus* soon became extinct after a possible host-plant switch[98,103,104].

## Possible Associations with Angiosperms

The presence of structurally distinctive *Cycadopites* pollen (Fig. 6) and five morphotypes of early angiosperm flowers (Supplementary Fig. 11) in Myanmar Amber are notable for their diminutive size compared to other, similar plant structures that typically are considerably larger in floral assemblages from other mid-Mesozoic intervals[97,105]. The micropyles of large seeds, often with unknown plant affinities, and ovulate reproductive structures containing tubes, channels, catchment funnels and pappus



**Supplementary Figure 11 │ *Tropidogyne* spp. and five angiosperm flower morphotypes with attached stamens from Myanmar amber.** (**a**), Flower CNU-PLA-MA-2015018 in dorsal view, left (2015018-1) is *Tropidogyne pikei* and right (2015018-2) is *Tropidogyne pentaptera*. (**b**), Flower CNU-PLA-MA-2015018-1 in polar view. (**c**), Flower CNU-PLA-MA-2015022 in lateral view, Morphotype A. (**d**), Flower CNU-PLA-MA-2015022 in polar view. (**e**), Flower CNU-PLA-MA-2015023 in polar view. (**f**), Flower CNU-PLA-MA-2015023 in dorsal view with enlargement of one stamen. (**g**), Flower CNU-PLA-MA-2015023 with four flowers in one amber piece, and enlargement of one flower in (**i**) with a clear stamen. (**h**), Flower CNU-PLA-MA-2015024 in lateral view. (**j**), Flower PAL 631404 in lateral view, Morphotype B. (**k**), Flower CNU-PLA-MA-2015047, a partly preserved generative shoot, Morphotype C. (**l**), Flower CNU-PLA-MA-2015026 in polar view. (**m**) and (**n**), Flower CNU-PLA-MA-2015029 in lateral view with two anthers in (**p**) and (**q**). (**o**), Several flowers from CNU-PLA-MA-2016001. (**u**), Flower CNU-PLA-MA-2017001 in lateral view. (**v**), Flower CNU-PLA-MA-2015028 in lateral view, Morphotype D. (**w**) to (**z**), Several flowers in CNU-PLA-MA-2016001 with three anthers illustrated in (**r**) to (**t**), all Morphotype E. Floral details are indicated in Supplementary Data 7. Scale bars represent 1 mm in (a)–(c), (g), (j), (k), (m), (w), (y) and (z); 0.5 mm in (d)–(f), (h), (i), (l), (n), (s), (u) and (v); 0.2 mm in (p) and (t), (r) and (t); 0.1 mm in (q); 2 mm in (x); and 5 mm in (o).

tubes[21,84] from which pollination drops were secreted are candidates for long-proboscid extraction of fluids by small-bodied insects such as Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae. A seemingly unlikely plant host would be rather small angiosperm flowers such as *Tropidogyne*[106,107] (Supplementary Fig. 11a,b,e–i,l–n,u), particularly as they lack a distinct tubular corolla. However, their cup-shaped form could have accommodated small, mosquito-size insects with long-proboscid aspect ratios, such as small moths, *Parapolycentropus* and *Dualula*. Based on their morphologies, these flowers likely secreted stigmatic fluids and perhaps bore floral nectaries to attract insects such as small beetles, thrips, parasitoid wasps, short-proboscid glossate moths, midges, labellate flies[94,96,101,108,109] and small scorpionflies. *Parapolycentropus* could have pollinated Morphotype E, a cup-shaped flower represented by four specimens (Supplementary Fig. 11r–t, w–z; Supplementary Data 6). Flower Morphotype A exhibits an average sepal length of 1.78 mm, although the distance from the sepal tip to the gynoecium would be somewhat shorter. The longest measured proboscis lengths of *P*. *burmiticus* is 1.53 mm (N=22) and *P*. *parapolycentropus* is1.50 mm (N=51), with no evident difference in male versus female proboscis lengths (Supplementary Data 4). These floral depths and proboscis lengths, taking into account a 0.9 mm difference resulting from the average sepal lengths minus the longest proboscis lengths, would allow elevation of the gynoecium above the floor of the corolla base, indicating an accommodating match (Supplementary Data 5 and 6). Additional pollinators may have associated with the more bowl-shaped flowers of *Tropidogyne pentaptera* with an average floral depth of 1.97 mm (N=5), and *T*. *pikei*, with a depth of 1.89 mm (N=3). We note that a possible angiosperm pollen grain adjacent a *Parapolycentropus* proboscis (Fig. 7e), suggests the presence of insect pollination.

*Dualula kachinensis* has a proboscis length that is considerably smaller and shorter than compression mesopsychid, aneuretopsychid and pseudopolycentropodid taxa (Supplementary Data 4), but larger and longer than both species of *Parapolycentropus* mentioned above (Supplementary Data 5). Considering that the single, measured complete proboscis of *D*. *kachinensis* is 3.23 mm, it would appear that none of the floral morphotypes – including Morphotype A, *T*. *pentaptera* and *T*. *pikei* – was sufficiently deep to accommodate the *D*. *kachinensis* proboscis (Supplementary Data 5 and6). The absence of an adequate fit indicates that *D*. *kachinensis* accessed pollination drops of other, probably gymnosperm, ovulate organs or possibly flowers with corollas approximately 1 mm deeper. Suspect candidates for access by *D*. *kachinensis* are *Samaropsis* ovules (micropyles), *Caytonia* fructifications (integumental tubes) and *Alvinia* cones (catchment funnels)[84,94,110–112], that occur in earlier compression deposits but may have had morphological analogs in Myanmar Amber. For *D. kachinensis*, the likely food was the nutritive liquid of gymnosperms and probably small, early angiosperm flowers with associations similar to small, modern moths on a variety of small angiosperm flowers[113]. Notably, the occurrence of Myanmar Amber with small, long-proboscid scorpionflies and early glossate moths, is at the end of the Aptian–Albian Gap, in which the shift from a gymnosperm to an angiosperm dominated global flora was well under way[98].

## Supplementary Note 5 | Hind-Wing Reduction in Mid-Mesozoic Insects, Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae

*Drosophila melanogaster* unquestionably has been the preeminent model organism for studies in evolutionary developmental biology for nearly the past 60 years[60,114]. During this time, regulatory mechanisms by homeotic containing genes (Hox genes) were known to play an important role the development of embryos. Simultaneously, there were studies of model insects, including those other than *D*. *melanogaster* that indicated features such as segmental identity and structures anatomically linked to particular segments, such as wings[115–119], were produced by the action of Hox genes on particular target sites of the organism[116]. Such Hox genes were highly conserved under normal conditions and typically had three primary consequences after their activation. One result was the change from one original structure to another, for example the transformation of a wing to a haltere or a leg. The other two functions of Hox genes were to suppress expression and detect and regulate structural change[117].

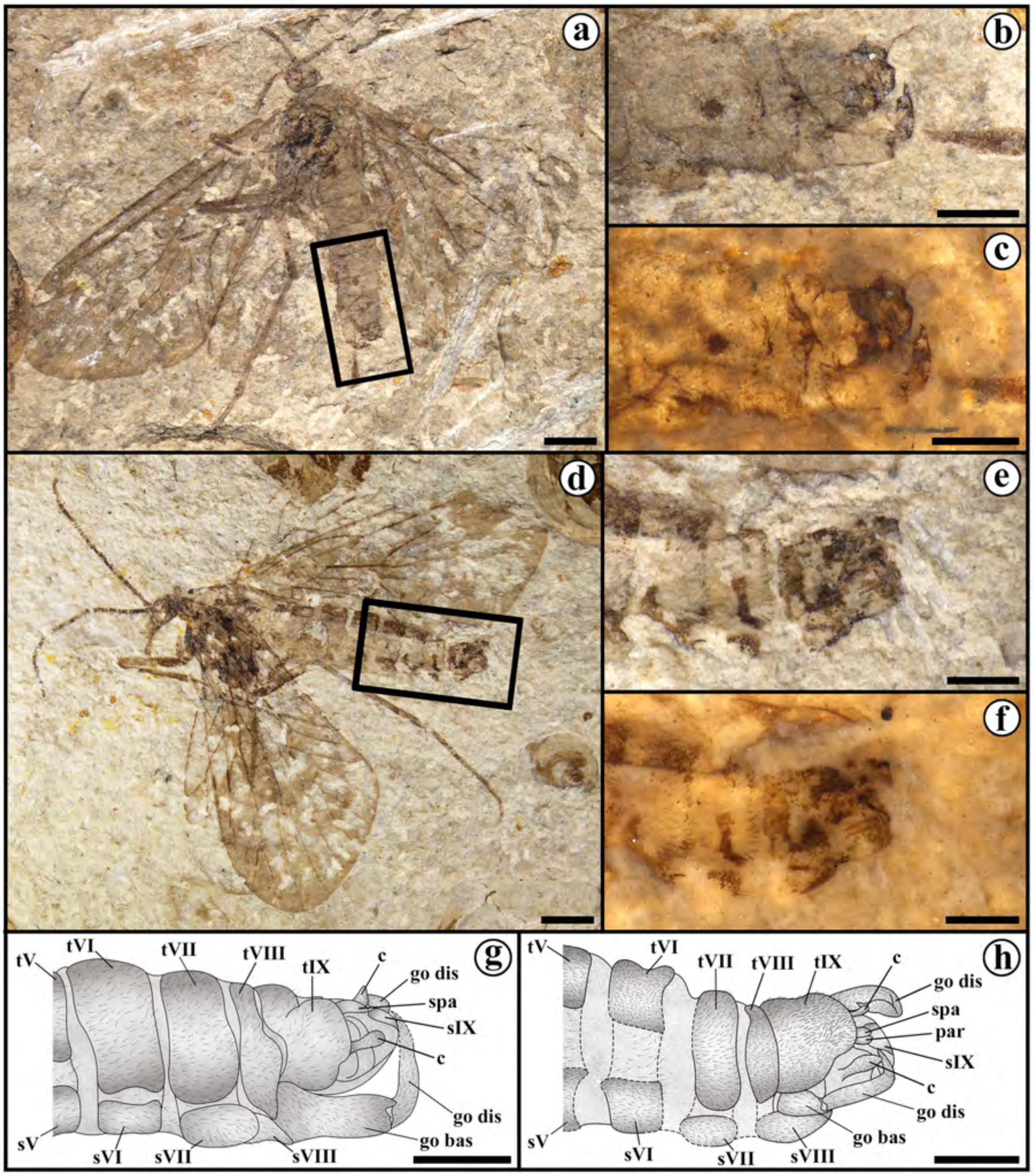
It was the first action, that two externally different but serially homologous structures could be changed into each other's phenotype, that became relevant for understanding how more subtler transformations, such as the change of a wing to a haltere would be developmentally possible. Among Hox-controlled genes, the *Ultrabithorax* (*Ubx*) gene could implement such a transformation of wings into halteres, and in the process suppress the development of a normal, membranous wing in *D. melanogaster*. Specifically, if the function of *Ubx* were removed in the metathoracic (T3) segment, halteres would develop into rather normal, membranous, fully veined wings[115,118]. Consequently, under blocked *Ubx* function and absent any intervening genetic changes, the transformation of wings to halteres is not attainable. In 2011, Pavlopoulos and Akam[118] designed experiments that would further address the morphological and molecular transformations after the inactivation of *Ubx* by induced temperature shift from 19℃ to 29℃. Their results revealed that ambient temperature is major factor that influences overall *Ubx* expression, and these changes not only happened in organ development but also at the cell level[118]. Additionally, this modulation by the *Ubx* gene has proved to target a particular developmental stage of the *D. melanogaster* embryo, and the switch from a normal wing to a haltere involves specification of other target genes in a complicated regulatory process.

Meanwhile, previous studies of insect flight biomechanics had indicated that dipterans were among the most agile of flying animals. This is a conclusion often attributed to typical functions of the modified hind wings (halteres) of Diptera. Early research by Pringle initially elucidated the flight dynamical mechanisms of the halteres in higher dipterans[119]. Thereafter, subsequent experiments using a variety of methods documented the role that halteres played in ensuring flight stability, agility and endurance by their function as gyroscopic sensors that provided neurosensory feedbacks to the forewings[120,121]. In pollination of flowers[84,105], gyroscopic control of flight suggests that a hovering position over gymnosperm reproductive organs occurred as they imbibed pollination drop fluids[89,94]. In addition, dipterans likely exhibited rapid and accurately relayed neural responses for high-speed body movements and large-angle, swerving manoevers[122,123], resulting in energetically efficient behaviors of plant foraging and evasion of predators. Owing to the haltere-like hind wings and small body size, early mecopterans would obtain advantages; for instance, greater sensitivity to airflow agitation in favor of making a rapid turns when eluding predators. During the mid Mesozoic, such advantages also became increasingly beneficial to larger, long-proboscid dipterans, such as rapidly hovering tanglevein flies (Nemestrinidae)[84]. The highly flickering wingbeat of tanglevein flies is indicated in their wings by anteriorly directed longitudinal veins[123] and their current pollinator interactions with deep-throated flowers.

Unfortunately, data from embryonic developmental experiments are lacking that would provide definitive evidence in extant mecopterans to support the conclusions from a variety of wing modifications we found in mid-Mesozoic Pseudopolycentropodidae, *Parapolycentropus* and *Dualula*. We hypothesize that these mid-Mesozoic long-proboscid lineages shared the same evolutionary developmental genetic mechanisms as modern *Drosophila*[116] and indeed all Diptera. It would be illuminating for future experimental studies to understand how these Hox-controlled genes regulate the formation of novel structures in modern Mecoptera[115]. Such explorations would provide a new evolutionary developmental perspective for understanding the change from a four-winged to a two-winged mecopteran.

## Supplementary Note 6 │ Genitalia structure in Mesopsychidae, Pseudopolycentropodidae, *Parapolycentropus* and Dualulidae

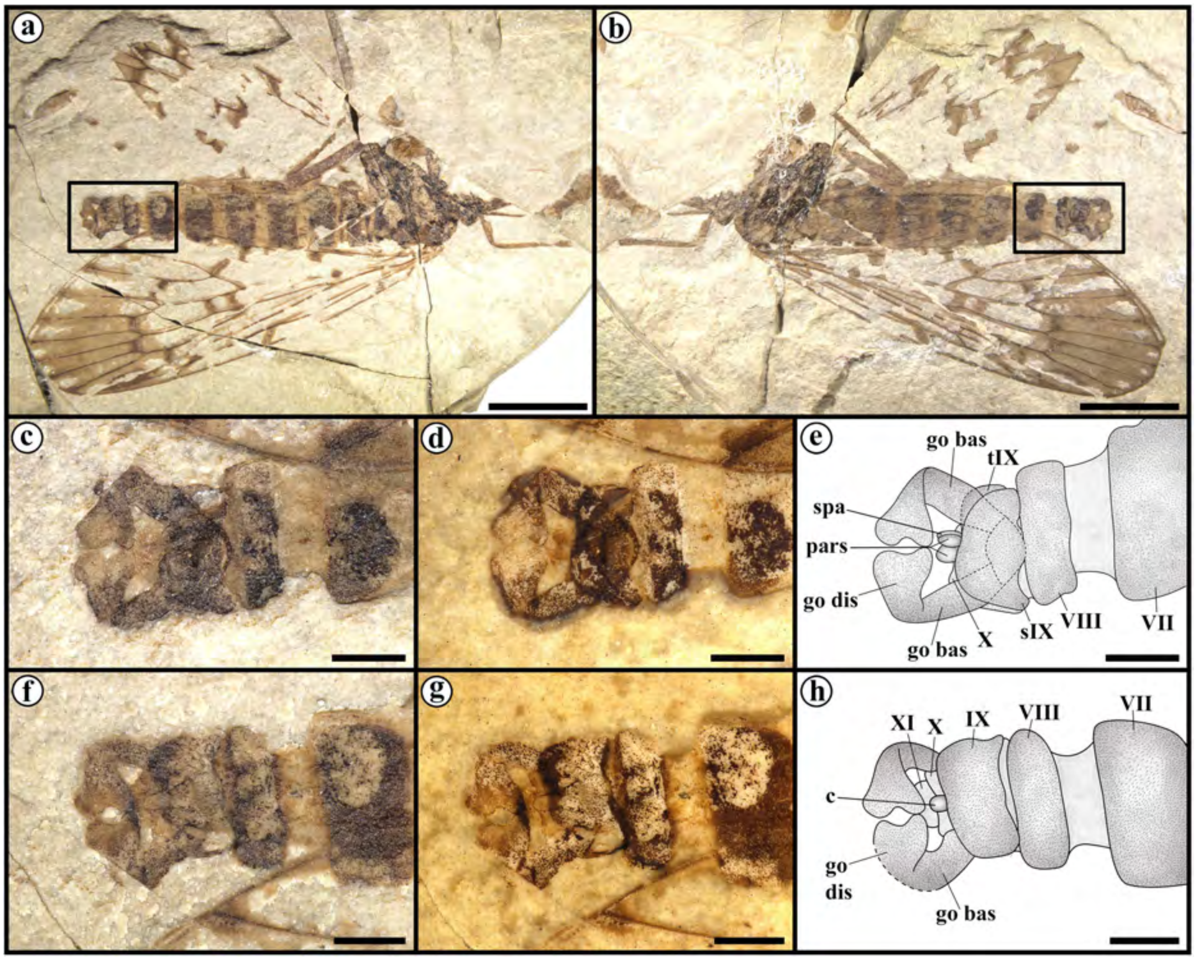
The male genitalia of Dualulidae (Supplementary Fig. 5c–e) and *Parapolycentropus*[69] (Figs. 6a,b; 7a,b; Supplementary Fig. 9a,b) are unique and significantly different from the male genitalia of most mecopterans (Supplementary Figs. 13,14) and all dipterans. A peculiarly distinctive feature is the upturned gonostylus (Supplementary Figs. 5c–e; 11g,h; and Fig. 8 in [69]).The male genitalia of extant



**Supplementary Figure 12 │ Male genitalia of *Pseudopolycentropus janeannae*[21,49].** Examined specimens are CNU-MEC-NN-2017050 (new material), and CUN-MEC-NN-2005004C, a paratype[21]. (**a**), Specimen of CNU-MEC-NN-2017050. (**b**), Male genitalia of (a) from template in (a). (**c**), Male genitalia of (b) under alcohol. (**d**), Specimen CNU-MEC-NN-2005004C. (**e**), Male genitalia from template in (d). (**f**), Male genitalia of (e) under alcohol. (**g**), Line drawing of the male genitalia from (b) and (c). (**h**), Line drawing of male genitalia from (e) and (f). Scale bars represent 1 mm in (a) and (d); 0.5 mm in (b), (c) and (e)–(h).

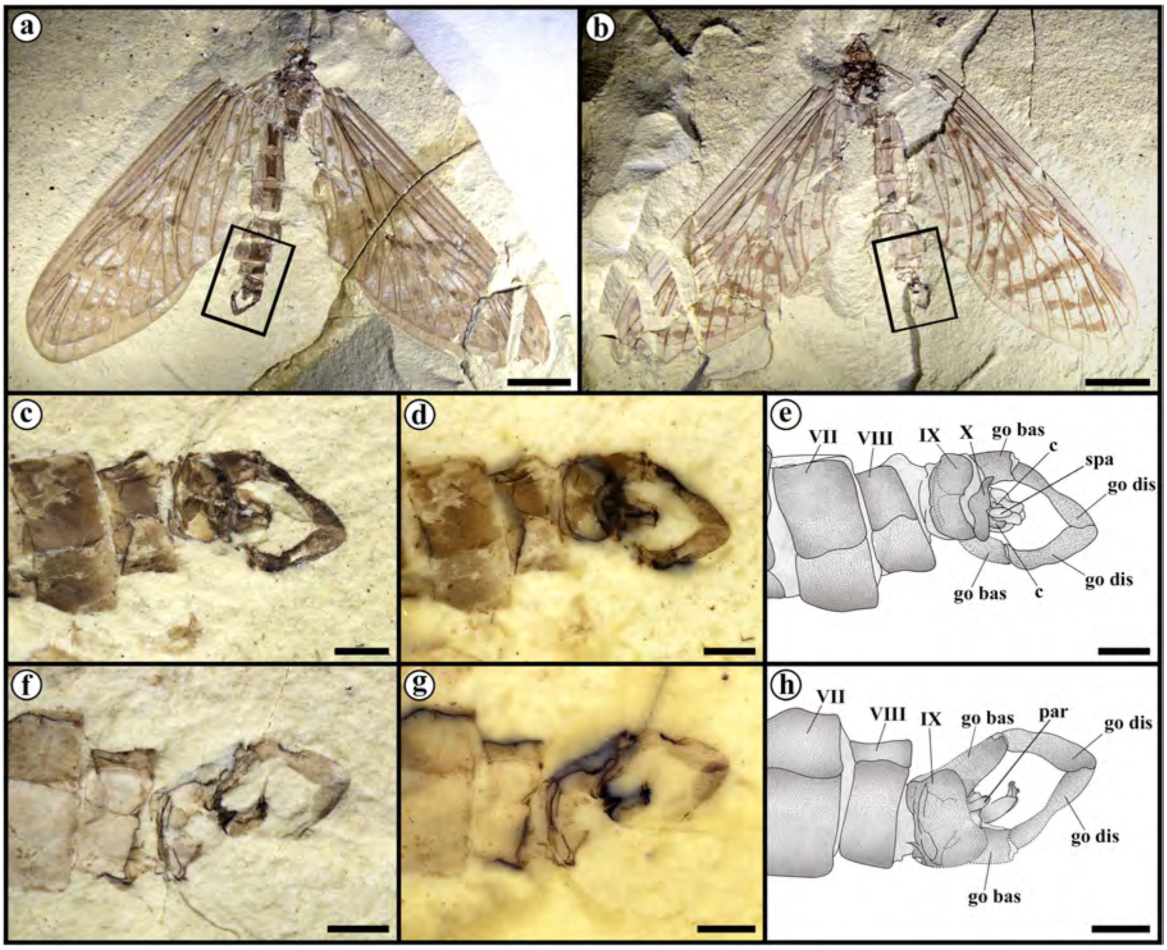
scorpionflies appear considerably different, and the most specialized groups such as Panorpidae[52,125], bear male genitalia that are enlarged and uplifted above the scorpionflies' dorsum, akin to the position of a scorpion metasoma and sting. However, for extinct taxa, most lineages lack well-preserved body structures. For instance, in the long-proboscid clade of Aneuretopsychina, Liassophilidae and Permotanyderidae[26,47,85,126,127], are the sister group to a Dualulidae + *Parapolycentropus* clade in the resulting trees (Fig. 1; Supplementary Fig. 1i). However, Liassophilidae and Permotanyderidae only include fossils preserved as compressions. Most constituent species are classified entirely on venational features[26,34,47,125–127]. In Aneuretopsychina, except Permian Nedubroviidae, the other three families have partly preserved abdominal features[21,39,56,57,83,87], but these records lack informative descriptions of genitalia.

We have reviewed all described specimens of Aneuretopsychina, evaluated relevant published papers and 37 new fossils of Mesopsychidae (15 specimens) and Pseudopolycentropodidae (22 specimens) from the CNU paleontological collections. We found four, clearly preserved, compression–impression fossils that bore relatively complete male genitalia. These new fossils were collected from Daohugou Village, Ningcheng County, of Inner Mongolia in Northeastern China. The fossils originate from the Jiulongshan Formation and are of latest Middle Jurassic age at 164 Ma[128,129]. From camera lucida



**Supplementary Figure 13 │ Male genitalia of *Lichnomesopsyche daohugouensis*[21,57].** The specimen is CNU-MEC-NN-2016019P/C, new material. (**a**), Specimen part. (**b**), Specimen counterpart. (**c**), Male genitalia from template in (a). (**d**), Male genitalia of (c) under alcohol. (**e**), Line drawing of male genitalia in (c) and (d). (**f**), Male genitalia outlined by template in (b). (**g**), Male genitalia of (f) under alcohol. (**h**), Line drawing of male genitalia from counterpart. Scale bars represent 5 mm in (a) and (b), and 1 mm in (c)–(h).

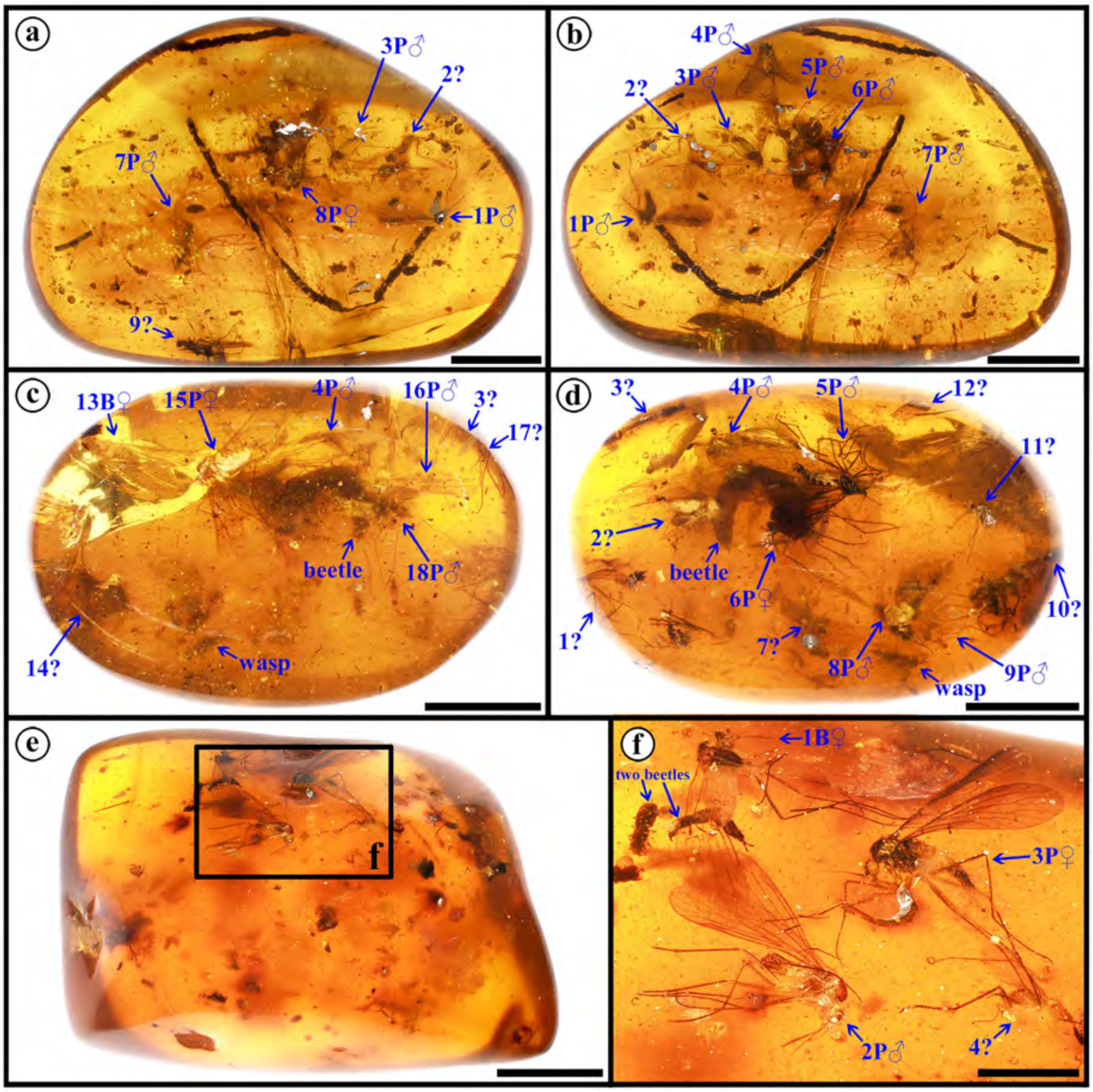
drawings and subsequent comparisons of the genitalic features of these specimens with other species of Aneuretopsychina, we found the most important similarities were claspers, each of which consisted of a basistylus (or gonocoxa) and a dististylus. In Mesopsychidae, the gonocoxa presents very thick and stout structures, especially for *Lichnomesopsyche daohugouensis*[21,57] (Supplementary Fig. 13). This species has a concavity on the tip of each dististylus, probably deployed for grasping a conspecific female abdomen during copulation. Another species of Mesopsychidae, *Epicharmesopsyche pentavenulosa*[87], bore claspers that were much longer and robust, but with a somewhat smaller concavity on the cusp of both dististyli (Supplementary Fig. 14). The male genitalia of *Parapolycentropus* in amber bore considerable general similarity to Pseudopolycentropodidae in compression fossils[49, 69] (Supplementary Fig. 12). The structures of these two last-mentioned taxa are quite similar, particularly the upwardly projecting dististylus and enlarged tIX tergum (Supplementary Fig. 14). An additional similarity is the presence of two ameristic cerci, one superanale and two paraprocts, exhibiting minimal differences in size and shape. Unrelated features, by comparison to other records of pseudopolycentropodids, are the two-winged mecopterans that have reduced sternites sII to sVI, indicating a structure associated with feeding on nutritious liquids[69]. In general, there were considerable, likely homologous, similarities among the male genitalia of Mesopsychidae, Pseudopolycentropodidae (including *Parapolycentropus*) and *Dualula*.



**Supplementary Figure 14 │ Male genitalia of *Epicharmesopsyche pentavenulosa***[87]. The specimen is CNU-MEC-NN-2015005P/C, new material. (**a**), Specimen part. (**b**), Specimen counterpart; **c**, Male genitalia outlined by template in (a). (**d**), Male genitalia of (c) under alcohol. (**e**), Line drawing of male genitalia in (c) and (d). (**f**), Male genitalia outlined by template in (b). (**g**), Male genitalia of (f) under alcohol. (**h**), Line drawing of male genitalia in (f) and (g). Scale bars represent 5 mm in (a) and (b), 1 mm in (c)–(h).

## Supplementary Note 7 | Reproductive Biology of *Parapolycentropus* and Dualulidae

Many insects engage in swarming behavior that is beneficial for congregation, mating and dispersal, particularly among Holometabola such as Hymenoptera, Lepidoptera, Trichoptera and Diptera[130]. The fossil record contains examples of swarming behavior, especially the social insects of termites, ants and bees[131–133], indicating that such behavior embodies a highly coordinated assembly of individuals gathering in a circumscribed site. The majority of fossil insect swarms consist of Diptera preserved in a variety of



**Supplementary Figure 15 | Three entombed swarms of *Parapolycentropus* spp.**[50,69] (**a**), Reverse view of six visible individuals in specimen CNU-MEC-MA-2016007. (**b**), Obverse view of seven visible individuals in (a) among nine total insects. (**c**), Reverse view of eight visible individuals in CNU-MEC-MA-2015038. (**d**), Obverse view of individuals in (c), with 12 individuals visible among 18 total *Parapolycentropus* individuals in the surrounding amber, including a beetle and a wasp. (**e**), Specimen CNU-MEC-MA-2015030. (**f**), Obverse view of individuals in (e), exhibiting the clustering of four *Parapolycentropus* individuals and two beetles. Abbreviations: **B**, *Parapolycentropus burmiticus*; **P**, *Parapolycentropus paraburmiticus*; and the question marks denote *Parapolycentropus* sp. and sex unknown. Scale bars represent 5 mm in (a), (b) and (e); 4 mm in (c) and (d); and 2 mm in (f).

ambers, with provenances from youngest to oldest, in the Dominican Republic, Baltic Region and associated Rovno area in Ukraine, Canada, France, Myanmar, Spain and Lebanon[131,132,134–136]. Modern groups of nematocerous Diptera, for example, Culicidae, Chironomidae and Ceratopogonidae; brachycerous Diptera including Bombyliidae; Tabanidae; Empididae and a few Cyclorrhapha such as Phoridae have been observed in mating flights[137]. One type of swarming reproductive behavior in dipterans is lekking, the participation in an airborne communal mating event[137]. However, this phenomenon rarely has been reported in extinct or extant mecopteran species, attributable, at least in modern groups, to scorpionflies requiring high levels of ambient food, or possessing a relatively narrow geographical distribution, or characterized by small population levels when compared to other Holometabola. Based on the imperfect preservational condition of most compression fossils, mecopteran lek events would be difficult to detect in the fossil record. Although lek events rarely are documented in the fossil record, the Nannochoristidae from the Middle Jurassic of Northeastern China[112] evidently is an exception.

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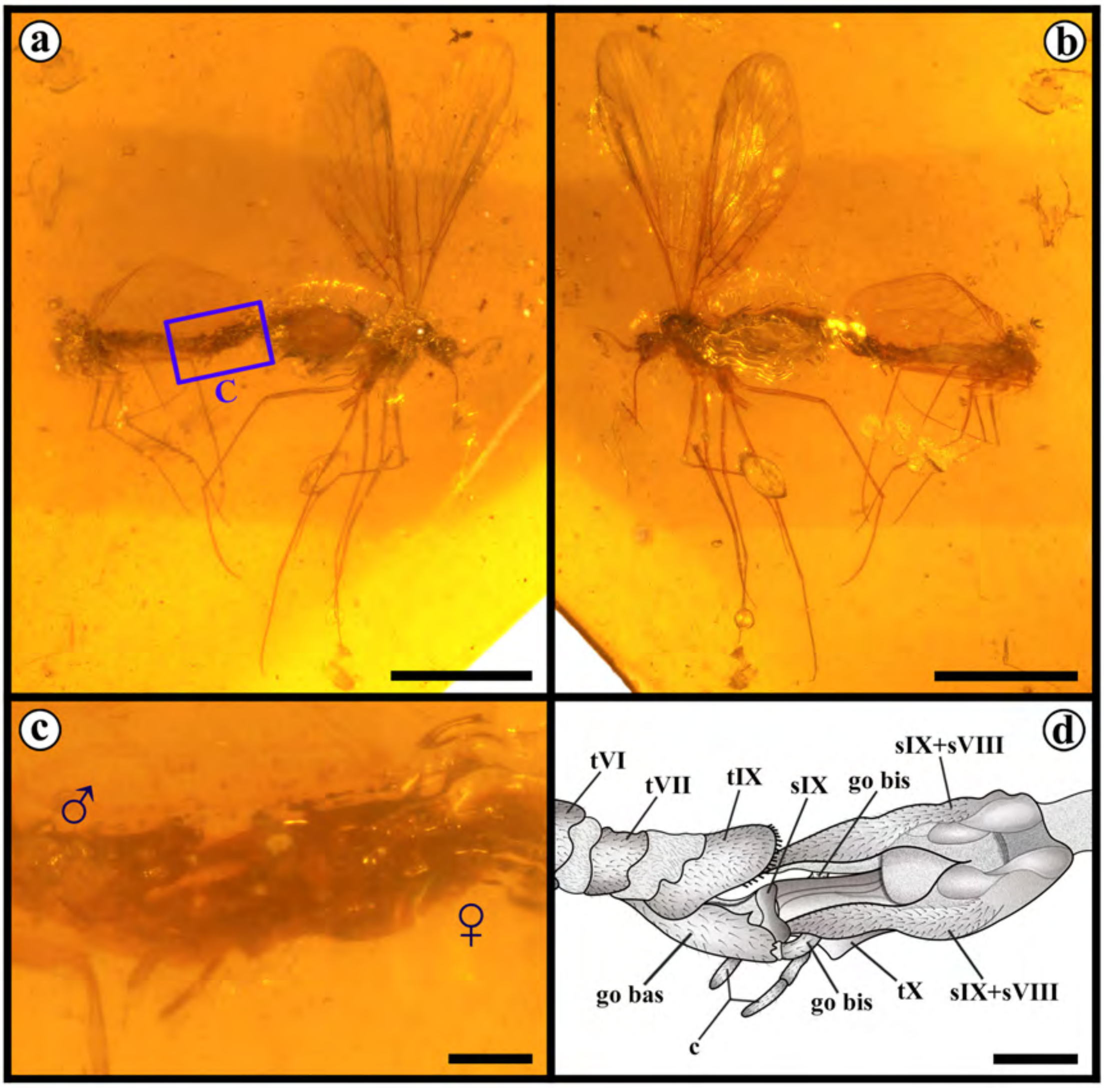

**Supplementary Figure 16 │ Copulating *Parapolycentropus paraburmiticus*[50].** These specimens show details of male and female genitalia (CNU-MEC-MA-2015025, new material). (**a**), Right lateral view. (**b**), Left lateral view. (**c**), Enlarged region of coupled male and female genitalia from the template in (a). (**d**), Line drawing showing details of mated terminalia, indicating inverted female genitalia. Scale bars: 2 mm in (a) and (b), and 0.2 mm in (c) and (d).

Even in amber, due to a limitation in the size of pieces, swarms of conspecific insect groups rarely are found. For *Parapolycentropus*, its body size is smaller than other known groups in Aneuretopsychina (Supplementary Data 4 and 5), and the presence of numerous individuals in an aggregation is more likely to be preserved in amber pieces of larger size. In 2014, an amber piece was reported that contained three male *Parapolycentropus* sp.[50,69], which is unlikely to record evidence of swarming behavior, as the insect number is too small for confirmation. Nevertheless, we found three additional amber pieces that consisted of abundant *Parapolycentropus* specimens with a variety of sex ratios. The first amber piece consisted of nine insects, all belonging to *Parapolycentropus paraburmiticus*[50], among them one female, six males, and two individuals of unknown sex (Supplementary Fig. 15a,b). The second amber piece was the richest one, and consisted of 18 *Parapolycentropus* sp. specimens assigned to three females, six males and nine of indeterminate sex, as well as a beetle and a small wasp, all clearly visible on both sides of the piece (Supplementary Fig. 15c,d). The third amber piece includes four scorpionflies, similar to the material in the 2014 paper[69]. This piece contains two females each of *Parapolycentropus paraburmiticus* and *Parapolycentropus burmiticus*, one male of *Parapolycentropus paraburmiticus*, one *Parapolycentropus* sp. of unknown sex, and two beetles (Supplementary Fig. 15e,f). The above amber pieces strongly suggest that these insects engaged in lekking behavior.

Additional evidence would be required to determine whether such assemblies represented a nuptial flight congregation, a mating lek or other informal gatherings. Many dipterans in mating swarms, or leks, appear to be composed mostly of males, informally termed male mating swarms, with females only periodically appearing in the lek and exiting soon after mating[130,138]. Moreover, smaller males are more likely to survive the selection process than larger ones, as they are easier to obtain one or more partners for copulation[139]. In *Parapolycentropus* and *Dualula*, males typically are smaller than females in body size but are more abundant in numbers when lekking. In the specimens mentioned above, excluding specimens of indeterminate sex, there is a definite imbalance in the male-to-female ratio. For example, the ratio of males and females in CNU-MEC-MA-2016007 is 6:1. Presently, there is evidence to indicate that some insect populations engaged in mating flight are composed overwhelmingly of males[30,138]. Nevertheless, due to an absence of sufficient fossil and modern samples, such evidence should serve as a reasonable hypothesis for further testing.

In many extant mecopterans, after the male offers a mating gift to attract the female, copulation ensues. The mating position is usually the female in the superior (upper) position, with the process controlled by the male. Simultaneously, the symmetric genitalia of the male and female are coupled, which is the primitive copulatory stance in Holometabola[139–141]. The best example of this juxtaposition of genitalia is Boreidae (snow scorpionflies), in which the male employs claspers and specialized wings to grasp the female[141,142]. However, there are some exceptions to this condition in modern mecopteran lineages, such as some Panorpidae (common scorpionflies), Panorpodidae (short-faced scorpionflies), Choristidae (Australian scorpionflies), and all Bittacidae (hangingflies). In modern *Panorpodes kuandianensis* (Panorpodidae), the male uses a pair of gonostyli to engage the female abdomen, and during copulation, the female reverses abdominal segments VII–IX by 180°[143]. There clearly are two or three phases during *P. kuandianensis* copulation. The first phase is the initial, V-shaped, side-by-side position with the abdomens converging at the V vertex. In the second phase, the shift to an end-to-end or tip-to-tip abdominal position is the final position in most individuals. This position often is modified by a third phase in some females with reversal of their body to ensure tight coupling with male genitalia[144].

The same copulation sequence exists in many Panorpidae as well[145,146]. Furthermore, in some mecopterans the male grasps the female wings or legs by notal or postnotal organs[145–147]. A few species form an O-shaped configuration in which the proboscis and genitalia of the male and female broadly connect with each other, most likely encouraged by males secreting a salivary mass as a nuptial gift to occupy the female throughout the copulation process[143]. This pattern also exists in extant Choristidae[148]. Bittacidae, however, are very different from other mecopteran groups in morphology and biology, and importantly during copulation the male and female are face-to-face in position, and the abdomen of the male twists ca. 180° to adapt to a pendant body position during copulation[149]. In early lineages of nematocerous and lower brachycerous Diptera, excluding specialized groups, almost all genitalia are symmetrical and exhibit several copulatory positions, including male-above, false male-above, general end-to-end, and targeted tip-to-tip positions[140]. The tip-to-tip position occurs during flight is typical of lekking behaviors. Dipteran examples of this in-flight, copulatory position include Chironomidae (nonbiting midges), and some Psychodidae (moth flies), Ceratopogonidae (biting midges) and Asilidae (robber flies), wherein the male temporary reverses abdominal segments 7 and 8 by about 180° to achieve contact with genitalia of the female[150–153].

Fortunately, one piece of Myanmar Amber entombed a copulating pair of *Parapolycentropus paraburmiticus*[50]. This distinctive interaction provides the first, direct evidence for understanding the mating behavior of mid-Mesozoic Aneuretopsychina. The amber piece is dark yellow, with many small-sized impurities of detritus, and contains numerous larger air bubbles surrounding the insect bodies. The male–female scorpionfly interaction documents many microscopic structures of the genitalia as well as a distinctive copulatory position (Supplementary Fig. 16). The male is significantly smaller than the female in body size and lacks preservation of its head and proboscis (Supplementary Fig. 16a,b). (We note that female decapitation of the head or feeding on the body of the male during copulation occasionally occurs in extant nematocerous dipterans[154], but in this case, the missing male head is a taphonomic in nature.) The copulating pair presents an obvious end-to-end, or tip-to-tip, position, with segments VII and VIII, and perhaps including part of segment VI of the female abdomen that twists 180° relative to its abdomen. The male uses its claspers to grasp female sIX and sVIII but due to the highly compact integration of genitalic segments, other bound structures may be obscured (Supplementary Fig. 16c,d). Such an unconventional juxtaposition of genitalia is very rare in Mecoptera; only a few Panorpodidae, a few Bittacidae and some Nannochoristidae, a relict clade, have the same pattern of end-to-end copulation[154,155]. Among this group, only panorpodids have adopted the mode of the female abdomen rotating rather than the male abdomen rotating, as illustrated in the position of the female genitalia relative to its terminal abdominal segments (Supplementary Fig. 16c,d). A similar mode of male–female genitalia position is present in basal groups of modern Diptera[154,156–158]. Based on the above data, *Parapolycentropus* very likely engaged in a mating flight behavior, probably as part of a swarm or lek, engaged in end-to-end copulation, with female genitalia in a reversed position.

**Supplementary Table 1 | Geological setting, locality, preservational status and sources for all described genera and species of Pseudopolycentropodidae.**

| Genus and species | Geological and locality | Preservational status | References |
|---|---|---|---|
| *Parapolycentropus burmiticus* | Middle Cretaceous, Kachin, Myanmar | Complete body with wings and mouthpart, male and female | [50, 69] |
| *Parapolycentropus paraburmiticus* | Middle Cretaceous, Kachin, Myanmar | Complete body with wings and mouthpart, male and female | [50, 69] |
| *Pseudopolycentropodes virginicus* | Late Triassic, Martinsville, USA | Incomplete body with wings, lack of mouthpart, sex unknown | [50] |
| *Pseudopolycentropus daohugouensis* | Middle Jurassic, Inner Mongolia, China | Incomplete body with wings and mouthpart, sex unknown | [49] |
| *Pseudopolycentropus janeannae* | Middle Jurassic, Inner Mongolia, China | Complete body with wings and mouthpart, male and female | [21,49] |
| *Pseudopolycentropus latipennis* | Late Jurassic, Karatau, Kazakhstan | Complete body with wings, female | [70, 71] |
| *Pseudopolycentropus madygenicus* | Late Jurassic, Fergana Valley, Kazakhstan | Incomplete forewing only, sex unknown | [71] |
| *Pseudopolycentropus novokshonovi* | Middle Jurassic, Inner Mongolia, China | Complete body with wings and mouthpart, female | [21,49] |
| *Pseudopolycentropus obtusus* | Upper Lias (Toarcian), Braunschweig, Germany | Incomplete forewing only, sex unknown | [72] |
| *Pseudopolycentropus perlaeformis* | Upper Lias (Toarcian), Dobbertin, Germany | Incomplete forewing only, sex unknown | [73] |
| *Pseudopolycentropus triangularis* | Upper Lias (Toarcian), Dobbertin, Germany | Complete fore- and hind wings | [74, 75] |
| *Pseudopolycentropus triasicus* | Middle Triassic (Ladinian), Arzviller, France | Incomplete forewing only, sex unknown | [76] |
| *Sinopolycentropus rasnitsyni* | Middle Jurassic, Inner Mongolia, China | Complete body with wings and mouthpart, female | [77] |

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