



OPEN

Phylogeny and biogeography of the enigmatic ghost lineage *Cylindrotomidae* (Diptera, Nematocera)

Iwona Kania-Kłosok¹✉, André Nel², Jacek Szwedo³, Wiktoria Jordan-Stasiło¹ & Wiesław Krzemiński⁴

Ghost lineages have always challenged the understanding of organism evolution. They participate in misinterpretations in phylogenetic, clade dating, biogeographic, and paleoecologic studies. They directly result from fossilization biases and organism biology. The *Cylindrotomidae* are a perfect example of an unexplained ghost lineage during the Mesozoic, as its sister family Tipulidae is already well diversified during the Cretaceous, while the oldest *Cylindrotomidae* are Paleogene representatives of the extant genus *Cylindrotoma* and of the enigmatic fossil genus *Cyttaromyia*. Here we clarify the phylogenetic position of *Cyttaromyia* in the stem group of the whole family, suggesting that the crown group of the *Cylindrotomidae* began to diversify during the Cenozoic, unlike their sister group Tipulidae. We make a comparative analysis of all species in *Cyttaromyia*, together with the descriptions of the two new species, *C. gelhausi* sp. nov. and *C. freiwaldi* sp. nov., and the revision of *C. obdurescens*. The cylindrotomid biogeography seems to be incongruent with the phylogenetic analysis, the apparently most derived subfamily Stibadocerinae having apparently a 'Gondwanan' distribution, with some genera only known from Australia or Chile, while the most inclusive *Cylindrotominae* are Holarctic.

Cylindrotomidae Schinner, 1863¹ together with Limoniidae Speiser, 1909², Pediciidae Osten-Sacken, 1860³, and Tipulidae Latreille, 1802⁴ sensu stricto are classified within Tipuloidea Latreille, 1802⁴, group of insects present in the fossil record since at least 220 Ma (Triassic)⁵. With only 71 extant⁶ and 16 extinct species⁷, this smallest family within Tipuloidea is divided into two subfamilies *Cylindrotominae*, represented mainly in Holarctic Region, and *Stibadocerinae* with an example of vicariant distribution with a sister-group relationship between South American and East Asian taxa, supporting hypothesis an 'ancestral' trans-Pacific biota⁸.

According to phylogenetic synthesis based on combined morphological characters of adult, larvae and pupae, together with nuclear gene sequence data as 28S rDNA or CAD, the *Cylindrotomidae* are found as a sister group of Tipulidae (both being treated as subfamilies in Tipulidae in Ref.⁹. This group of insects is generally indicated as a sister group or being closely related to the Tipulidae by other authors^{10–20}. But, within *Cylindrotomidae*²¹, only the representatives of subfamily *Cylindrotominae* are known from fossil record. The oldest described representatives of *Cylindrotominae* are only known from the Paleogene (56.0–47.8 Ma) by the extant genera *Cylindrotoma* Macquart, 1834²² and *Diogma* Edwards, 1938^{23–33}, plus the extinct genus *Cyttaromyia* Scudder, 1877^{7,34}, while the oldest stem Tipulidae are Jurassic and the oldest crown Tipulidae are Cretaceous^{5,35–37}. Thus, the *Cylindrotomidae* can be considered as a typical ghost lineage during the Mesozoic.

This phytophagous group of craneflies, which immatures lives among mosses and herbaceous plants.

The *Cylindrotomidae* (Supplementary Data S1) probably knew a period of diversification during the Eocene, sufficient to become frequent enough to be found as fossils. Most of the fossil *Cylindrotomidae* are

¹Department of Biotechnology, Institute of Biology and Biotechnology, University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, Poland. ²Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, Université des Antilles, EPHE, Paris, France. ³Laboratory of Evolutionary Entomology and Museum of Amber Inclusions, Department of Invertebrate Zoology and Parasitology, University of Gdańsk, 59, Wita Stwosza St, 80308 Gdańsk, Poland. ⁴Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland. ✉email: ikania@univ.rzeszow.pl

know from the Middle Eocene Baltic amber^{31,32,38,39} and the Late Eocene Florissant Formation in USA^{24,39}. Three species of *Cylindrotoma* are known from impressions of the Ypresian Fur Formation; two species from the Ølst Formation of Denmark were described within *Cyttaromyia*²⁹ (Supplementary Table S1). Four species of *Cyttaromyia* were described from the Eocene Green River Formation USA, the other were described from Florissant Formation and Kishenehn Formation in USA, Middle Salt Formation in Alsace (France), Biamo Formation in Russia, and from Baltic amber^{24,27,28,30,31,40}. Here we propose a morphological phylogenetic analysis to define the relationships between extinct genus *Cyttaromyia* and the other taxa in the family. We also describe two new species of *Cyttaromyia* on the basis of new fossils from the same Formation, and new technics of research give us possibility to redescribe *Cyttaromyia obdurescens* Cockerell, 1924²⁷.

Results

Systematic paleontology.

Order Diptera Linnaeus, 1758⁴²

Infraorder Tipulomorpha Latreille, 1802⁴

Family Cylindrotomidae Schinner, 1863¹

Subfamily Cylindrotominae Schinner, 1863¹

Genus *Cyttaromyia*

Type species: *C.yttaromyia fenestrata* Scudder, 1877³⁴, by monotypy.

Key to species of the genus *Cyttaromyia* Scudder, 1877³⁴

1. Wings without distinct patterning.....3.
 - Distinct patterns of coloration on wings.....2.
2. Rs longer than R_{2+3+4} and R_{3+4} combined.....
 -*Cyttaromyia vahldieki* Freiwald, 1991²⁹
Denmark/Ølst Formation
 - Rs shorter than R_{2+3+4} and R_{3+4} combined.....
 -*Cyttaromyia rayona* Freiwald & Krzemiński, 1991³⁰
Russia/Biamo Formation
3. Wings hyaline.....4.
 - Wings pale brownish without conspicuous markings with end of marginal cell apically somewhat clouded (Cockerell, 1924).....
 -*Cyttaromyia reclusa* Cockerell, 1924²⁶
USA/Green River Formation/Roan Moutains/Colorado
4. Vein R_1 well-developed.....5.
 - Vein R_1 reduced.....7.
5. Crossvein m-cu situated beyond bifurcation of Mb on M_{1+2} and M_{3+4} ; Sc terminating in C far beyond fork of Rs.....6.
 - Crossvein m-cu situated at bifurcation of Mb on M_{1+2} and M_{3+4} ; Sc terminating in C just beyond fork of Rs.....
 -*Cyttaromyia gelhausi* sp. nov.
USA/Green River Formation
6. Crossvein sc-r one of its length before tip of Sc; A_1 tip before tip of Sc level.....
 -*Cyttaromyia obdurescens* Cockerell, 1925²⁷
USA/Green River Formation/Roan Moutains/Colorado
 - Crossvein sc-r at least two of its length before tip of Sc; A_1 tip beyond e tip of Sc level.....
 -*Cyttaromyia princetoniana* Scudder, 1894²⁴
USA/Green River Formation
7. Sc elongate, terminating in C well beyond level of fork of Rs.....8.
 - Sc short, terminating in C before level of fork of Rs.....
 -*Cyttaromyia freiwaldi* sp. nov.
USA/Green River Formation

8. Crossvein m-cu situated at most or before fork of Mb on M_{1+2} and M_{3+4}9.
 – Crossvein m-cu situated beyond fork of Mb on M_{1+2} and M_{3+4}
*Cyttaromyia frelloi* Krzemiński, 1998³¹
 Baltic amber
9. Vein r-r (R_2) terminating before level of r'-m', before level of m-m.....10.
 – Vein r-r (R_2) terminating at level of r'-m', behind level of m-m.....
*Cyttaromyia fenestrata* Scudder, 1877³⁴
 USA/Green River Formation
10. d'-cell as long as d-cell or shorter.....11.
 – d'-cell longer than d-cell.....12.
11. M_{3+4} bifurcation on M_3 and M_4 approximately at level of m-m; d-cell 2× as long as M_4 ; m-m beyond level of tip of r-r (R_2).....
*Cyttaromyia lynnae* De Jong, 2019⁴⁰
 USA/Kishenehn Formation.
- M_{3+4} bifurcation on M_3 and M_4 before level of m-m; d-cell 1.5× as long as M_4 ; m-m approximately at level of tip of r-r (R_2).....
*Cyttaromyia fuscula* Cockerell, 1921²⁵
 USA/Green River Formation
12. Fork of Mb at level of fork of Rs; d'-cell narrowed at base.....
*Cyttaromyia quievreuxi* Séguy, 1934²⁸
 Alsace, France/Middle Salt Formation.
- Fork of Mb before level of fork of Rs; d'-cell narrow, but not narrowed at base.....13.
13. Tip of r-r (R_2) beyond level of tip of fork of M_{3+4} on M_3 and M_4 ; Rs at 2× as long as R_{2+3+4}
*Cyttaromyia scudderi* Freiwald, 1991²⁹
 Denmark/Ølst Formation
- Tip of r-r (R_2) before level of tip of fork of M_{3+4} on M_3 and M_4 ; Rs at approximately as long as R_{2+3+4}
*Cyttaromyia rossi* Krzemiński, 2019⁴¹
 UK/Isle of Wight/Bembridge Marls

Cyttaromyia obdurescens Cockerell, 1925²⁷
 (Fig. 1).

Material examined. Holotype No. 26284 (AMNH) (female); American Museum National History; Green River Formation USA, Eocene.

Emended diagnosis. Wing without color spots; Sc elongate, terminating in C well beyond level of fork of Rs, beyond r-m level but far before m-m and r'-m' level; opposite approximately half the length of R_{2+3+4} ; vein r-r (R_2) terminating far before r'-m' and m-m level, at level of basal part of M_3 ; R_1 well-developed; R_{2+3+4} longer than half length of Rs; d'-cell longer than d-cell, narrowed at its base; crossvein m-cu positioned beyond fork of Mb on M_{1+2} and M_{3+4} ; apical section of M_3 almost as long as d-cell; A_1 tip positioned near apex of wing, far behind level of Mb bifurcation on M_{1+2} and M_{3+4} , before r-m level.

Comparison. *Cyttaromyia obdurescens* differs from *C. fenestrata*, *C. freiwaldi* sp. nov., *C. frelloi*, *C. fuscula*, *C. lynnae*, *C. quievreuxi*, *C. scudderi*, and *C. vahldieki* by a well-developed vein r-r (R_2). Wing of *C. obdurescens* is without spot. *C. rayona* and *C. vahldieki* have different patterning of wings. In contrast to *C. gelhausi* sp. nov., crossvein m-cu is situated beyond Mb, while in *C. gelhausi* sp. nov., it is located at Mb bifurcation, in *C. frelloi* just before Mb bifurcation. In *C. obdurescens*, vein sc-r is located one of its length from tip of Sc, tip of A_1 is located before tip of Sc, while in *C. princetoniana* vein sc-r is located at least two of its length before the tip of Sc, A_1 tip is located beyond level of tip of Sc. In *C. obdurescens* crossvein m-cu is positioned beyond fork of Mb measured from base of wing, while in *C. rossi*, m-cu is distinctly before fork of Mb.

***Cyttaromyia gelhausi* sp. nov.** <http://zoobank.org/urn:lsid:zoobank.org:act:9165E3D2-514B-4524-9F85-A9895CBF2A31>.

(Figs. 2, 3).

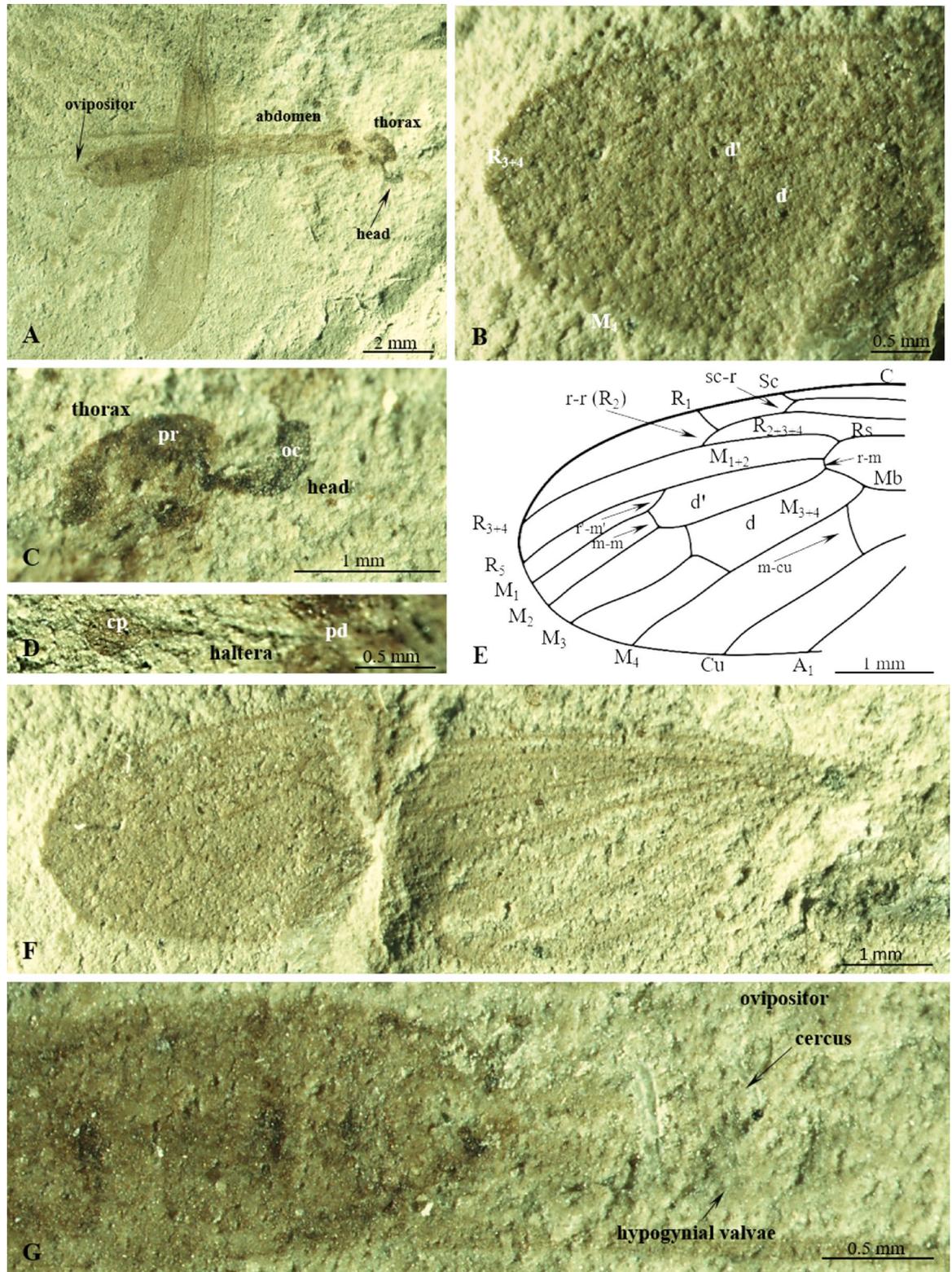


Figure 1. *Cyttaromyia obdurescens* Cockerell, 1925²⁷, holotype No. 26284 (AMNH) (female): (A) habitus, lateral view; (B) apex of wing; (C) head and thorax, enlarged lateral view; (D) haltera; (E) apex of wing, drawing; (F) wing; C. terminal part of abdomen with *ovipositor* visible. cp, *capitellum*, pd, *pedicellus* of haltera, oc, *ocellus*, ped, *pedicellus*, pr, *pronotum*.

Material examined. Holotype MNHN.FA71341 (18a, male); additional material 18b, female; 18c, female (on the same slab with holotype); Muséum national d’Histoire naturelle (MNHN), Paris; Green River Formation USA, Eocene.

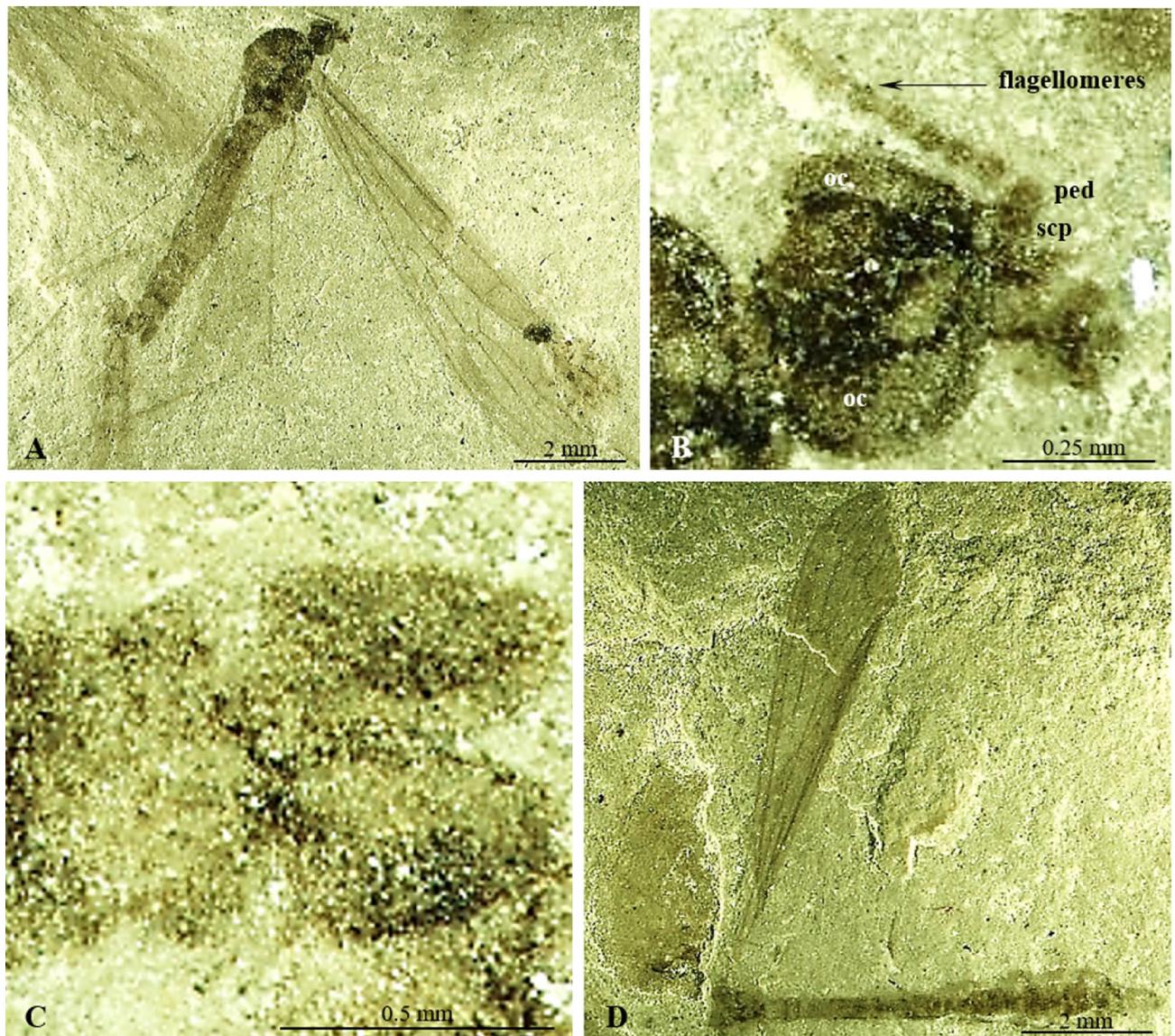


Figure 2. *Cyttaromyia gelhausi* sp. nov.: A.–C. No. MNHN.FA71341 (18a, male) (holotype): (A) habitus, latero-ventral view; (B) head, ventral view; (C) hypopygium, ventral view; D. No. MNHN.FA71341 (18c, female) (additional material), habitus, lateral view. oc, ocellus; ped, pedicellus; scp, scapus.

Etymology. The specific name is given to honor Doctor John Gelhaus (Academy of Natural Sciences of Drexel University), the eminent specialist on extinct and extant insects.

Diagnosis. Flagellomeres short and relatively wide; wing without color spots; Sc not very elongate, terminating in C just beyond level of fork of Rs, opposite level of crossvein r–m; opposite approximately 1/10 length of R_{2+3+4} ; vein r–r (R_2) terminating far before r'–m' and just before m–m level, at level of basal part of M_3 ; R_1 well-developed; R_{2+3+4} longer than half length of Rs; d'-cell longer than d-cell, narrowed at its base; crossvein m-cu positioned at fork of Mb on M_{1+2} and M_{3+4} ; apical section of M_3 almost as long as d-cell.

Comparison. *Cyttaromyia gelhausi* sp. nov. has no distinct color patterning of the wings in contrast to *C. vahldieki* and *C. rayona*. The wing of *C. reclusa* is pale brownish without conspicuous markings²⁶, but the end of the marginal cell and the veins bounding the discal cell apically are somewhat clouded. The body length of *C. gelhausi* sp. nov. is at most 6.83 mm, wing length 7 mm, while the body length of *C. reclusa* is 13.5 mm and wing length 12 mm. In *C. gelhausi* sp. nov. tibial spurs are absent while pattern of tibial spurs of *C. frelloi* is 1:1:2³¹. Vein R_1 is well-developed in *C. gelhausi* sp. nov., while in *C. freiwaldi* sp. nov., *C. frelloi*, *C. fuscata*, *C. lynnae*, *C. quievreuxi*, *C. rossi*, *C. scudderi*, *C. vahldieki* R_1 is reduced. In *C. gelhausi* sp. nov. Sc is not very elongate, terminating in C just beyond level of fork of Rs, opposite the level of crossvein r–m, while in *C. fenestrata*, *C. frelloi*, *C. fuscata*, *C. lynnae*, *C. obdurescens*, *C. princetoniana*, *C. quievreuxi*, *C. rayona*, *C. scudderi*, and *C. vahldieki*, it is terminating far beyond level of Rs. In *C. freiwaldi* sp. nov. Sc terminating in C before Rs level. In *C. fenestrata* and *C. vahldieki*, Sc is very elongated, terminating in C opposite basal part of M_3 . Crossvein m-cu is positioned

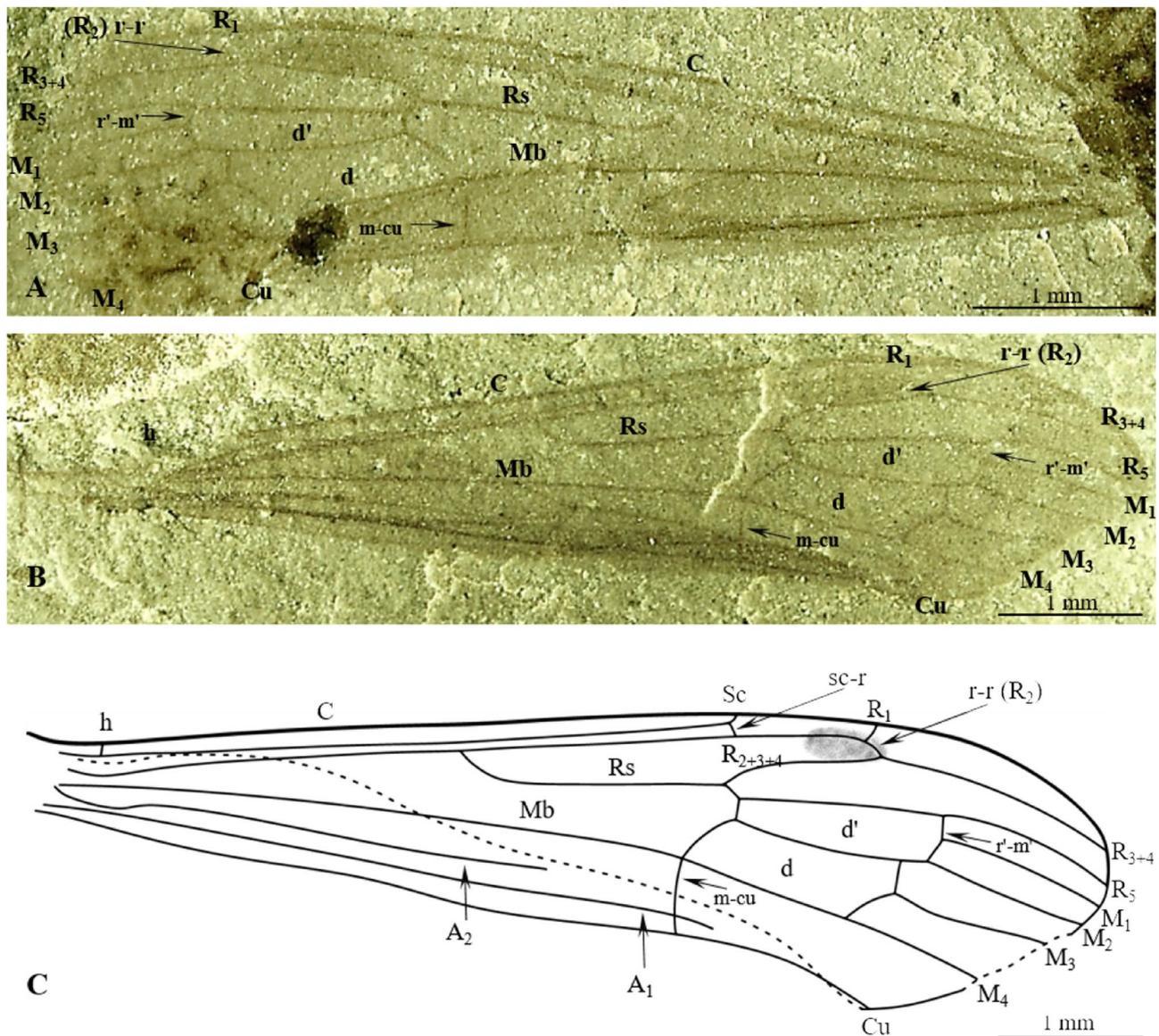


Figure 3. *Cyttaromyia gelhausi* sp. nov.: (A) No. MNHN.FA71341 (18a, male) (holotype), wing; (B), C. No. MNHN.FA71341 (18c, female) (additional material): (B) wing; (C) wing, drawing.

at fork of Mb on M₁₊₂ and M₃₊₄ while in other fossil species of the genus *Cyttaromyia* this vein is situated beyond fork of Mb; in *C. frelloi* this vein is located before fork of Mb. Moreover, in *C. vahldeiki* and *C. fenestrata* vein r-r (R₂) terminating in R₃₊₄ beyond d' level, far beyond the level of basal part of M₃, in *C. gelhausi* sp. nov. this vein is terminating at the level of d', at the level of basal part of M₃.

***Cyttaromyia freiwaldi* sp. nov.** (Figs. 4, 5).

<http://zoobank.org/urn:lsid:zoobank.org:act:DB604450-AD47-4644-82E0-A33FEAEB7157>.

Material examined. Holotype MNHN.FA71342 (70A, part/68, counterpart, female), additional material 70B, female, on the same slab as holotype), Muséum national d'Histoire naturelle (MNHN), Paris; Green River Formation USA, Eocene.

Etymology. The new species is dedicated to the German eminent researcher Doctor Andre Freiwald (Institut für Paläontologie, Universität Erlangen).

Diagnosis. Wing without color spots; Sc short, terminating in C before level of fork of Rs, far before level of crossvein r-m; vein r-r (R₂) terminating far before r'-m' level and at m-m level, just beyond level of basal part of M₃; R₁ atrophied; R₂₊₃₊₄ longer than half length of Rs; d'-cell shorter than d-cell, narrowed at its base; crossvein m-cu positioned beyond fork of Mb on M₁₊₂ and M₃₊₄; M₃ shorter than d-cell.

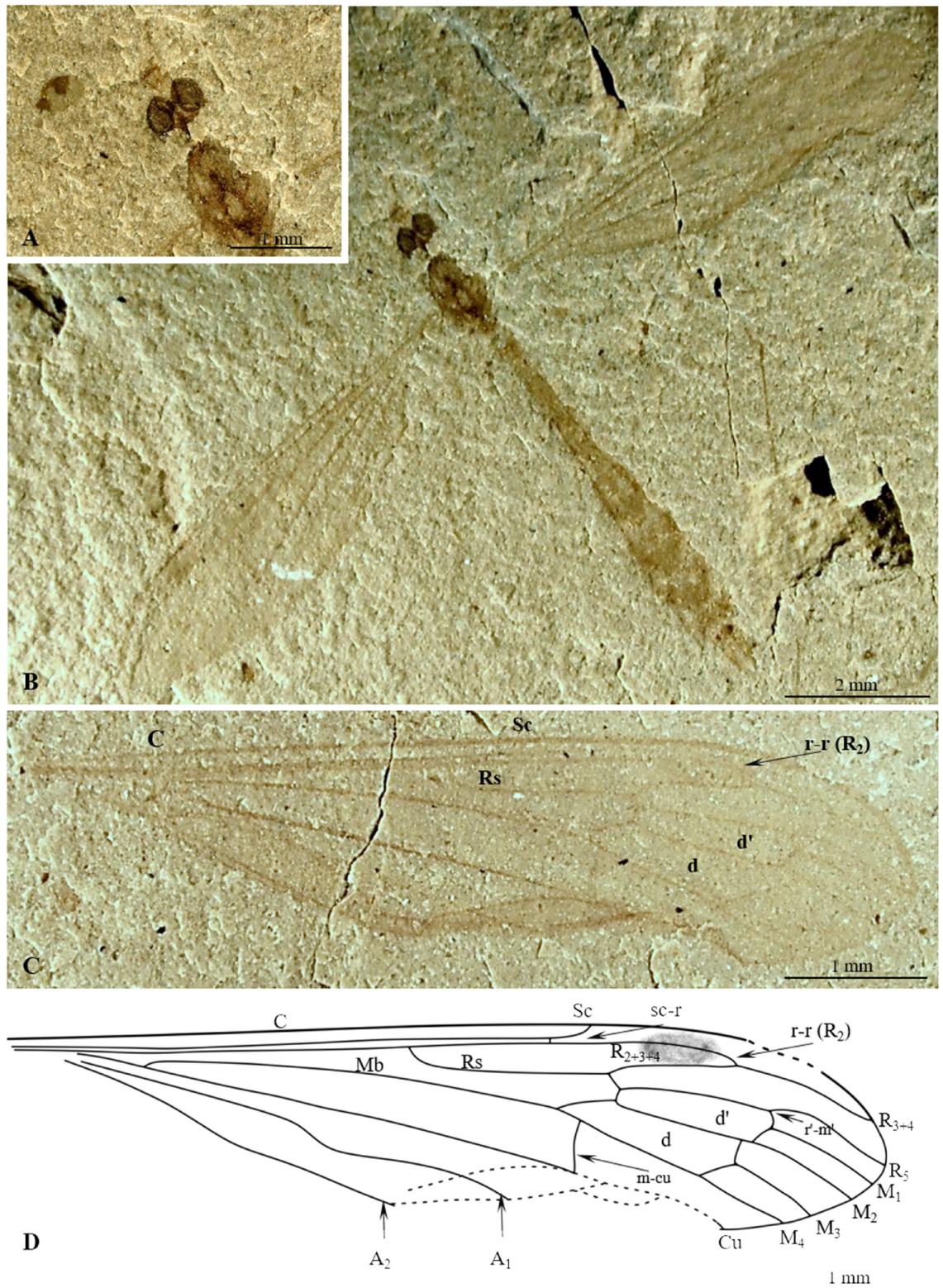


Figure 4. *Cyttaromyia freiwaldi* sp. nov. No. MNHN.FA71342 (70A, part, female), (holotype): (A) habitus, dorsal view; (B) head, dorsal view; (C) ovipositor, dorsal view, (D) wing venation, (E) wing venation, drawing.

Comparison. *Cyttaromyia freiwaldi* sp. nov. has no distinct patterning of the wings in contrast to *C. vahldieki* and *C. rayona*. In contrast to *C. reclusa*, the body length of *C. freiwaldi* sp. nov. is at most 4.8 mm, wing length 6.22 mm, while the body length of *C. reclusa* is 13.5 mm and wing is 12 mm long with the end of the marginal cell and the veins bounding the discal cell apically are somewhat clouded²⁶. *C. freiwaldi* sp. nov. differs from other

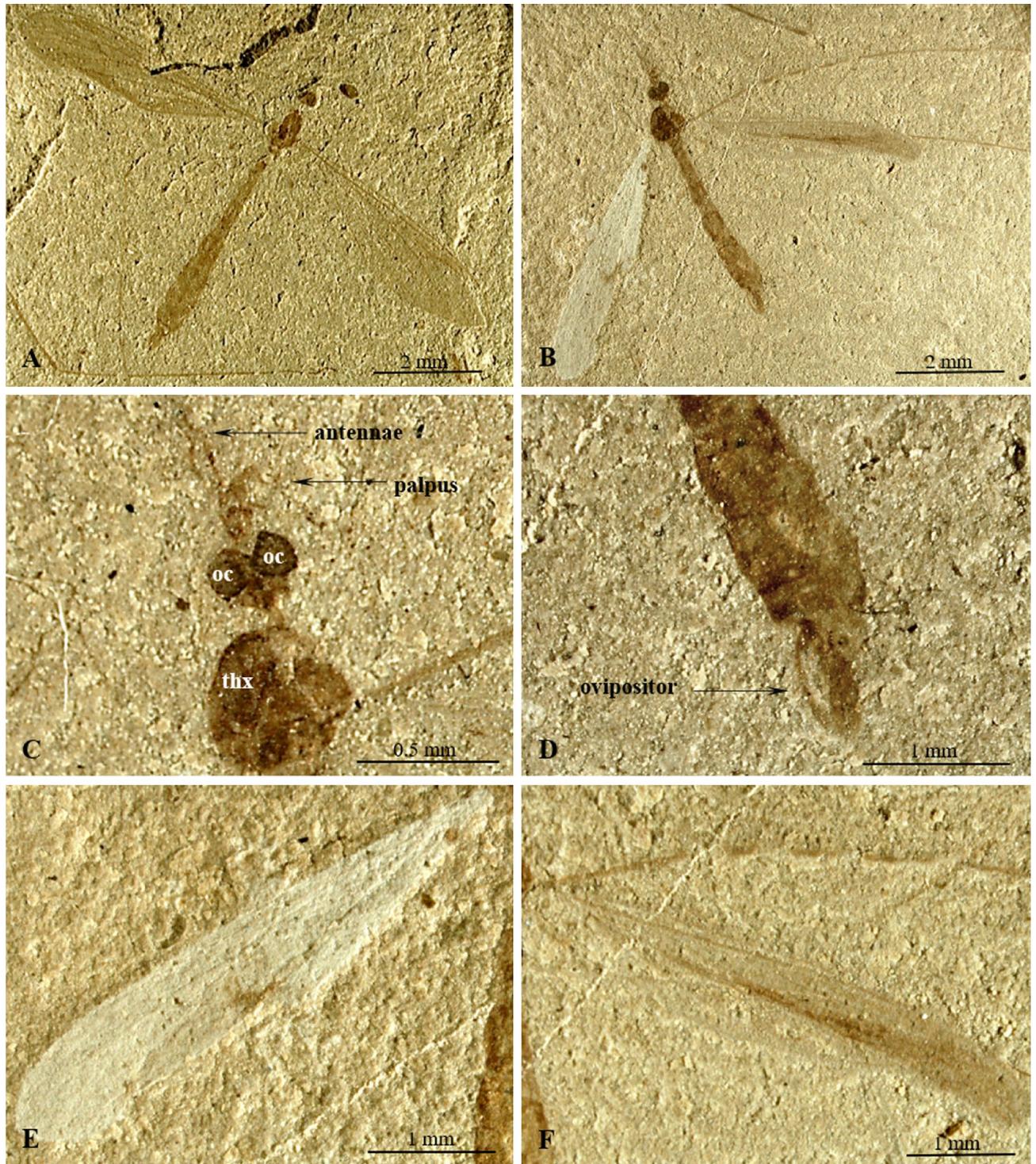


Figure 5. *Cyttaromyia freiwaldi* sp. nov.: (A) No. MNHN.F.A71342 (68, counterpart, female), (holotype), habitus, dorsal view; B.-F. No. MNHN.F.A71342 (70AB, female), (additional material): (B) habitus, dorsal view; (C) head, dorsal view; (D) terminal part of abdomen with ovipositor visible, dorsal view; (E) left wing; (F) right wing. oc, ocelli; thx, thorax.

fossil species especially by point of termination of Sc. In *C. freiwaldi* sp. nov. vein Sc terminating in C before fork of Rs while in other fossil species, excluding *C. reclusa*²⁷, Sc terminating in C just beyond or far beyond bifurcation of Rs. Moreover, in contrast to *C. gelhausi* sp. nov., *C. obdurescens*, *C. princetoniana*, and *C. rayona*, the vein R_1 in *C. freiwaldi* sp. nov. is reduced. Vein $r-r$ (R_2) of *C. freiwaldi* is terminating in R_{3+4} at d-cell level while in *C. fenestrata* and *C. rayona* beyond this level, in *C. vahldieki* even beyond d'-cell level (Supplementary Data S2).

Phylogenetic position of *Cyttaromyia* within *Cylindrotomidae*. The parsimony analysis yielded three equally most parsimonious cladograms, 53 steps long, with consistency index CI=62, RI=66. Their consensus majority rule cladogram is shown in Fig. 6A. The *Cylindrotomidae* clade is supported by six synapomorphies: presence of petiole (character 15, state 0), relationship of R_3 and R_4 (character 17, state 1), position of crossvein m-cu relative to the bifurcation of M_{3+4} (character 18, state 1), shape of d-cell (character 20, state 1), position of tip of A_2 (character 25, state 0), morphology of aedeagus (character 28, state 2). *Cyttaromyia* is supported on consensus tree by the 'presence of supernumerary crossvein connecting vein R_{4+5} with M_1 near its origin, to produce two discal cells' (character 16, state 1). The clade (*Cylindrotominae* + *Stibadocerinae* (Hennig, 1973)⁴³) is supported by two synapomorphies, relationship of M_1 and M_2 (character 14, state 1), position of tip of A_2 (character 26, state 0). The clade [*Phalacrocerca replicata* Linnaeus, 1758⁴¹ + (*Liogma nodicornis* Osten Sacken, 1865⁴⁴ + *Triogma trisculata* Shummel, 1829⁴⁵)] is supported by one synapomorphy, viz. position of crossvein m-cu relative to the bifurcation of Mb (character 19, state 1). *Triogma trisculata* appears as the sister-group to *Liogma nodicornis*, the clade (*Liogma nodicornis* + *Triogma trisculata*) being supported by three synapomorphies: shape of flagellomeres (character 2, state 1), position of R_5 (character 13, state 1), degree of reduction of crossvein r-m (character 24, state 1). The clade *Stibadocerinae* [= (*Stibadocerodes australiensis* Alexander, 1922⁴⁶ + (*Stibadocera bullans* Enderlein, 1912⁴⁷) + (*Stibadocerella pristina* Brunetti, 1918⁴⁸ + *Stibadocerina chilensis* Alexander, 1928⁴⁹))] is supported by two synapomorphies: the number of branches of R_s reaching wing margin (character 9, state 1), relationship of R_3 and R_4 (character 17, state 2) (Supplementary Data S3).

Discussion

The subfamily *Cylindrotominae*²¹ currently contains more species and genera, compared to the *Stibadocerinae*. The oldest record of the extinct genus *Cyttaromyia* is Paleogene, as for the genus *Cylindrotoma*. The fossil record of the *Cylindrotominae* dated back to at least 56.0 Ma²⁹, with no evidences on older occurrences. Other representatives of *Cylindrotominae* are known in the Eocene^{24,25,27,28,31,32,38}, but most of them are strictly modern⁶ (Supplementary Figs. S1, S2).

According to our parsimony analysis (consensus tree), *Cyttaromyia* falls as sister group of all the extant genera of *Cylindrotomidae*, and thus belongs to the stem group of the family, and could correspond to a different subfamily. Also, the extant *Cylindrotominae* appear paraphyletic in respect to the *Stibadocerinae* because the two genera *Cylindrotoma* and *Diogma* fall in an unresolved polytomy with this subfamily plus a clade that contains the other *cylindrotomine* genera. Nevertheless, these results are preliminary and would need to be completed by the addition of characters, in particular molecular.

The *Cylindrotominae* (and also *Cyttaromyia*) have a Holarctic distribution, while the *Stibadocerinae* have a more disjunctive distribution in Indo-Malaysia, Australo-Papua and Southern Neotropics (Taiwan, China, Indonesia, Malaysia, India, Papua New Guinea, Philippines, Australia, and Chile). Such distribution resembles that of an ancient Gondwana group, with 'relic' taxa in Australia and Chile; but the present phylogenetic analysis would contradict this hypothesis, as the only known stem representative of the family is also Holarctic. Further analyses together with discoveries of fossil *Cylindrotomidae* in the Southern Hemisphere shall be necessary to clarify this complex, strange situation.

From a taxonomic point of view, *Architipula* is characterized by the occurrence of vein Sc tip beyond fork of R_s level, subequal to or a little shorter than veins R_{2+3} and R_3 combined, distinctly inclined crossvein m-m between M_{1+2} and M_3 , usually short and straight vein A_2 ⁵¹. *Cyttaromyia* is characterized by the occurrence of two discal cells (d-cell and d'-cell), supernumerary crossvein r'-m' connecting vein R_5 with M_1 near its origin, to produce two discal cells. Some similarities are present in the wing venations of *Cyttaromyia* and *Cylindrotoma*: separate M_1 and M_2 and relatively long vein M_1 . In *Cylindrotoma* the crossvein r'-m' is atrophied, but the base of vein M_1 is strongly arched and only one discal cell (d-cell) is present. In all other *Cylindrotominae*, like *Diogma* (recorded from the Middle Eocene)³², or other genera with a younger fossil record, the crossvein r'-m' is reduced and only one discal cell (d-cell) is present (Fig. 6B,C; Supplementary Figs. S3, S4).

Conclusion

The revision of *Cyttaromyia obdurescens* and the description of two new species *Cyttaromyia gelhausi* sp. nov. and *Cyttaromyia freiwaldi* sp. nov., allowed us to propose a key to the species of this genus. We have also made the first morphological phylogenetic analysis of the *Cylindrotomidae*, with in the rather surprising result of the putative paraphyly of the *Cylindrotominae* and a position of *Cyttaromyia* in the stem group of this family.

Material and methods

The study was based on material from the collection of the Muséum national d'Histoire naturelle (MNHN), Paris (five specimens) and American Museum National History (AMNH) (one specimen). The imprints from sediments of Green River Formation USA (age 50.3–46.2 Ma⁴²) were studied using a Nikon SMZ 1500 stereomicroscope equipped with a Nikon DS-Fi1 camera in University of Rzeszów. The microphotographs and measurements were taken with NIS-Elements D 3.0 software. Drawings were completed by tracing the photographs, nomenclature of wing venation was used³¹.

The Eocene Green River Formation USA (50.3–46.2 Ma) (Rocky Mountains, Colorado, Wyoming, Utah) is one of the most famous Eocene palaeontological sites of the World. The sediments include mainly calcium carbonate, calcite and aragonite. The occurrence of different types of sludge varies with the geological levels. Tipton Shale Member in Greater Green River Basin is the oldest rock formation of the Green River⁵².

Placement of the genus *Cyttaromyia* within *Cylindrotomidae* was tested with the use of Maximum Parsimony (MP) criterion, implemented in TNT 1.5 software package, with the 'Traditional Search' options^{53,54}, with memory to store 99,999 trees, 10,000 replications, with 100 trees to save per replication; utilizing

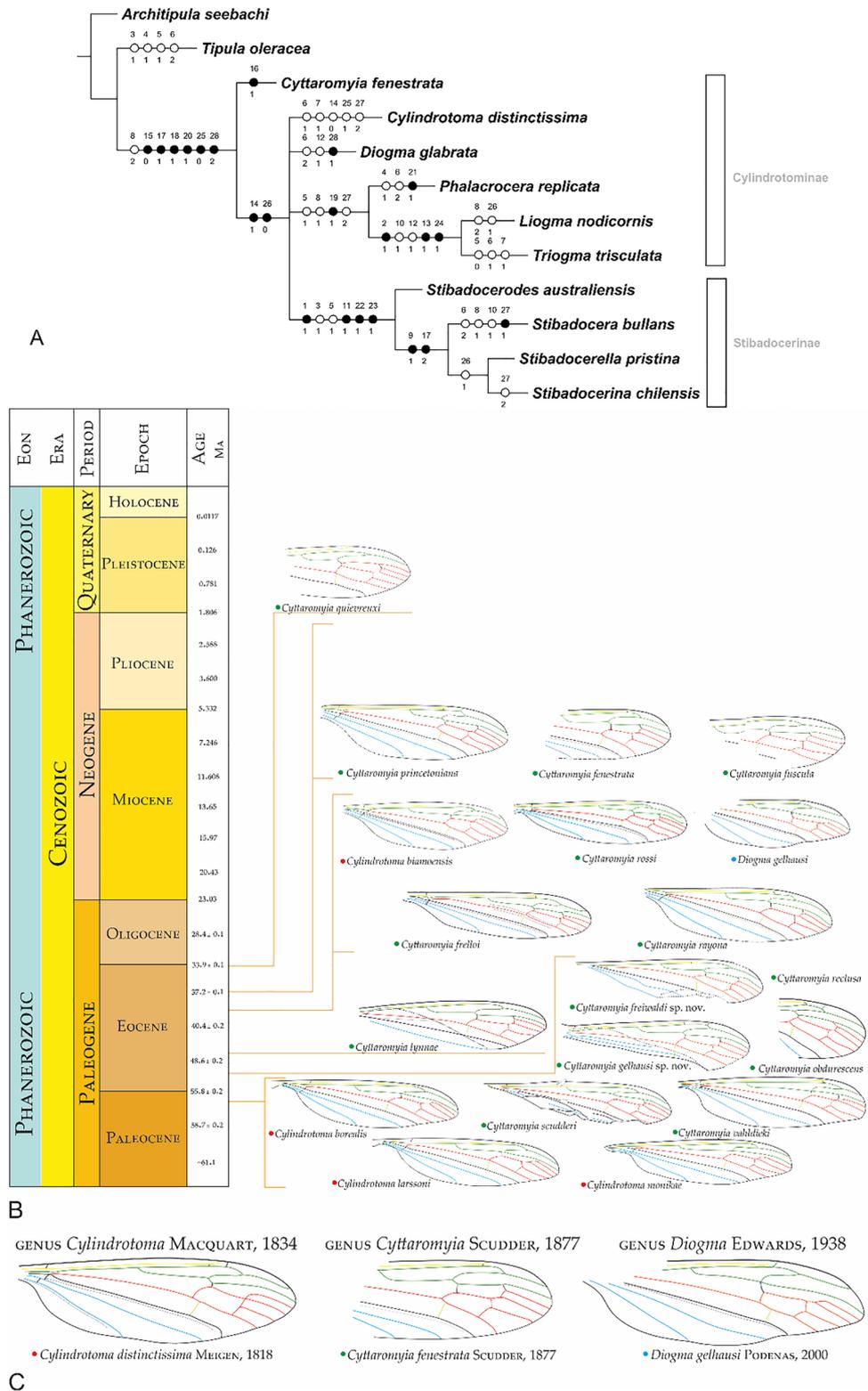


Figure 6. (A) Consensus relationships tree of genera of subfamily Cylindrotominae. Filled circles indicate synapomorphies or autapomorphies; open circles indicate plesiomorphies. Number of character given above circles, states of characters below circles; (B) Wing venation of fossil Cylindrotominae with chronostratigraphic distribution view; (C) Wing venations of representatives of genera: *Cylindrotoma*, *Cyttaromyia*, *Diogma*, represented in fossil record. Wing venation redrawing^{24,25,27–29,31,34,42,50}. Stratigraphic chart according to International Stratigraphic Chart, International Commission of Stratigraphy (v. 2021/05) <https://stratigraphy.org/chart>.

tree-bisection-reconnection (TBR) algorithm and collapsing zero length branches. The type species of extinct and extant genera of family Cyndrotomidae were included in the analysis. *Architipula seebachi* (Geinitz, 1884)⁵⁵—type species of the genus *Architipula* Handlirsch, 1906⁵⁶, was selected as outgroup because the Architipulinae are closely related to Cyndrotominae. *Tipula oleracea* Linnaeus, 1758⁴¹ was used as a type species of the genus *Tipula*, in the family Tipulidae, currently considered as the sister family of the Cyndrotomidae. The morphological data to the matrix were compiled in the Nexus file using Mesquite v. 3.61 build 927⁵⁷. All 28 characters of the imagines used in the analysis were treated as unordered and unweighted (Supplementary Table S2). Equal weighting analysis (EW) was performed⁵³; the trees received were viewed and their features studied using WinClada 1.00.08 and ASADO 1.61, with Unambiguous Changes Only, Fast Optimization (ACCTRAN) and Slow Optimization (DELTRAN) options^{57–59}. Tree files received were adjusted using Corel Draw X3 and Photo-Paint Software. The 28 morphological characters of the imago observed in the fossil and recent material and used for analysis are listed below. The data matrix given is partly based on used morphological features^{8,21,34,41,43–45,50,56}.

Received: 28 January 2021; Accepted: 31 May 2021

Published online: 06 July 2021

References

- Schiner, J. R. Vorläufiger Commentar zum dipterologischen Theile der Fauna austriaca. V [concl.]. *Wiener Entomologische Monatschrift* **7**, 217226 (1863).
- Speiser, P. 4 Orthorapha. Orthorapha Nematocera. Wissenschaftliche ergebnisse der Schwedischen zoologischen expedition nach dem Kilimandjaro, dem Meru und den umgebenden Massaissteppen Deutsch-Ostafrikas 1905–1906, unter leitung von prof. dr. Yngve Sjostedt. *Diptera* **10**, 31–65 (1909).
- Osten Sacken, C. R. New genera and species of North American Tipulidae with short palpi, with an attempt at a new classification of the tribe. *Proc. Acad. Nat. Sci. Phila.* **1859**, 197–254 (1860).
- Latreille, P. A. Histoire naturelle, generale et particuliere, des Crustaces et des Insectes. Tome troisieme. Ouvrage faisant suite a l'histoire naturelle generale et particuliere, composee par Leclerc de Buffon, et redigee par C.S. Sonnini, membre de plusieurs societes savantes. *Familles naturelles des genres, Paris*, 13–467. I–xii (1802).
- Krzemiński, W. *Tipula* (s. lato) *eva* n. sp. from Cretaceous (East Asia)—The oldest representative of the family Tipulidae (Diptera, Polyneura). *Acta Zool. Crac.* **35**, 43–44 (1992).
- Oosterbroek, P. Catalogue of the Crane-flies of the World. (Diptera: Tipuloidea: Pedicidae, Limoniidae, Cyndrotomidae, Tipulidae). <https://ccw.naturalis.nl/> Accessed 28 May 2020 (2020).
- Evenhuis, N. L. Catalog of the fossil flies of the world (Insecta: Diptera) website. Version. 2.0. <http://hbs.bishopmuseum.org/fossilcat/> Accessed 13 Feb 2014 (2014).
- Ribeiro, G. C. The Neotropical genus *Stibadocerina* Alexander and its phylogenetic relationship to other Stibadocerinae genera: Further evidence of an ancestral trans-Pacific biota (Diptera: Cyndrotomidae). *Syst. Entomol.* **34**, 324–333 (2009).
- Petersen, M. J., Bertone, M. A., Wiegann, B. M. & Courtney, G. W. Phylogenetic synthesis or morphological and molecular data reveals new insights into the higher level classification of Tipuloidea (Diptera). *Syst. Entomol.* **35**, 526–545 (2010).
- Alexander, C. P. Notes on the genus *Dicranoptycha* Osten Sacken (Tipulidae, Diptera). *Entomol. News* **30**, 19–22 (1919).
- Alexander, C. P. New or little-known Tipulidae (Diptera). III. Ethiopian species. *Ann. Mag. Nat. Hist.* **9**(5), 465–472 (1920).
- Savchenko, E. N. Tipulidae. *Fauna Ukrainy* **14**, 1–551 (1966).
- Savchenko, E. N. Limoniidae of South Primorye. *Akademiï Nauk Ukrainskoy SSR, Kiev* 1–156 (1983).
- Brodo, F. A revision of the genus *Prionocera* and the phylogeny of the family Tipulidae (Diptera). PhD Dissertation, *Carleton University, Ottawa, Ontario* (1984).
- Oosterbroek, P. & Theowald, B. Phylogeny of the Tipuloidea based on characters of larvae and pupae (Diptera, Nematocera), with an index to the literature except Tipulidae. *Tijdschrift voor Entomologie* **134**, 211–267 (1991).
- Starý, J. Phylogeny and classification of Tipulomorpha, with special emphasis on the family Limoniidae. *Acta Zool. Crac.* **35**, 11–36 (1992).
- Ribeiro, G. C. The phylogeny of the Limnophilinae (Limoniidae) and the early evolution of the Tipulomorpha (Diptera). *Invertebr. Syst.* **22**, 627–694 (2008).
- Zhang, X. *et al.* Comparative Mt genomics of the Tipuloidea (Diptera: Nematocera: Tipulomorpha) and its implications for the phylogeny of the Tipulomorpha. *PLoS ONE* **11**(6), 1–20 (2016).
- Lukashovich, E. D. & Ribeiro, G. C. Mesozoic fossils and the phylogeny of Tipulomorpha (Insecta: Diptera). *J. Syst. Paleontol.* **17**, 635–652 (2019).
- Kang, Z., Zhang, X. & Yang, D. Characterization of the complete mitochondrial genome of the snow crane-fly *Chionea crassipes gracilistyla* (Diptera, Tipuloidea, Limoniidae) with phylogenetic analysis. *Mitochondrial DNA (B)* **4**, 2662–2663 (2019).
- Osten Sacken, C. R. Monographs of the Diptera of North America. Part IV. *Smithson. Misc. Collect.* **8**(219), 345 (1869).
- Macquart, P. J. M. Histoire naturelle des insectes. Diptères. Tome première. *N.E. Roret Paris*. 578 (1834).
- Edwards, F. W. On the British Lestremiinae, with notes on exotic species—3. (Diptera, Cecidomyiidae). *Proc. R. Entomol. Soc. Lond. (B)* **7**, 102–108 (2009).
- Scudder, S. H. Tertiary Tipulidae, with special reference to those of Florissant, Colorado. *Proc. Am. Philos. Soc.* **32**, 163–245 (1894).
- Cockerell, T. D. A. Eocene insects from the Rocky Mountains. *Proc. U.S. Natl. Mus.* **57**, 233–260 (1921).
- Cockerell, T. D. A. Fossil insects in the United States National Museum. *Proc. U. S. Natl. Mus.* **64**(13), 1–15 (1924).
- Cockerell, T. D. A. Plant and insect fossils from the Green River Eocene of Colorado. *Proc. U. S. Natl. Mus.* **66**(19), 1–13 (1925).
- Séguy, E. Un nouveau Cyndrotomine fossile (Tipulidae). *Encycl. Entomol. (B)* **II**(7), 47–48 (1934).
- Freiwald, A. Insekten aus der Fur-Formation von Dänemark (Moler, oberes Paleozän/ unteres Eozän). 5. Cyndrotomidae (Diptera: Tipulomorpha). *Meyniana* **43**, 97–123 (1991).
- Freiwald, A. & Krzemiński, W. Cyndrotomidae (Diptera, Tipulomorpha) from the Paleogene of Bolshaya Svetlovodnaya (eastern Asiatic USSR). *Paläontol. Z.* **65**, 339–344 (1991).
- Krzemiński, W. *Cyttaromyia frelloi* sp. n., the first representative of the family Cyndrotomidae in Baltic amber (Diptera: Tipulomorpha). *Polskie Pismo Entomologiczne* **67**, 303–308 (1998).
- Podenas, S. A new species of *Diogma* Edwards, 1938 (Diptera, Cyndrotomidae) from Baltic amber (Eocene). *Trans. Am. Entomol. Soc.* **126**(1), 103–107 (2000).
- Brodo, F. A review of the subfamily Cyndrotominae in North America (Diptera: Tipulidae). *Univ. Kansas Sci. Bull.* **47**, 71–115 (1967).
- Scudder, S. H. The first discovered traces of fossil insects in the American tertiaries. *Bull. U. S. Geol. Geogr. Surv. Territ.* **3**, 741–762 (1877).

35. Krzemiński, W. & Ansoerge, J. New Upper Jurassic Diptera (Limoniidae, Eoptychopteridae) from the Solnhofen lithographic limestone (Bavaria, Germany). *Stuttgarter Beiträge zur Naturkunde (B)* **221**, 1–7 (1995).
36. Lukashovich, E. D. Limoniidae (Diptera) in the Upper Jurassic of Shar Teg, Mongolia. *Zoosymposia* **3**, 131–154 (2009).
37. Krzemiński, W., Kopeć, K. & Kania, I. New and little known species from the genus *Leptotarsus* Guerin-Meneville, 1831 (Diptera: Tipulidae) from the Lower Cretaceous of Northern Brazil. *Cretac. Res.* **78**, 103–108 (2017).
38. Loew, H. Ueber den Bernstein und die Bernsteinfauna. *Programm der K. Realschule Meseritz* 1–44 (1850).
39. Krzemiński, W. Revision of the fossil Cylindrotomidae (Diptera, Nematocera) from Florissant and White River, USA. *Paläontol. Z.* **65**, 333–338 (1991).
40. Krzemiński, W. *et al.* True flies (Insecta: Diptera) from the late Eocene insect limestone (Bembridge Marls) of the Isle of Wight, England, UK. *Earth Environ. Sci. R. Soc. Edinb.* **110**, 495–554 (2019).
41. Linnaeus, C. *Systema Naturae per Regna tria Naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. T. 1. Holmiae: Impensis Direct. Laurentii Salvii.* 800 (1758).
42. Greenwalt, D. E. *et al.* Diptera of the middle Eocene Kishenehn Formation. I. Documentation of diversity at the family level. *Palaeontol. Electron.* **22**, 1–56 (2019).
43. Hennig, W. Flügelgeäder und System der Dipteren unter Berücksichtigung der aus dem Mesozoikum beschriebenen Fossilien. *Beiträge zur Entomologie* **4**, 245–388 (1973).
44. Osten Sacken, C. R. Description of some new genera and species of North American Limnobia. Part I. *Proc. Entomol. Soc. Phila.* **4**, 224–241 (1865).
45. Schummel, T. E. Beschreibung der in Schlesien einheimischen Arten einiger Dipteren-Gattungen I Limnobia. Meigen. *Beiträge zur Entomologie, besonders in Bezug auf Schlesien, Breslau* **1**, 97–201 (1829).
46. Alexander, C. P. New or little-known Tipulidae (Diptera) IX. Australasian species. *Ann. Mag. Nat. Hist.* **9**(9), 297–315 (1922).
47. Enderlein, G. 1912. Die Phoridenfauna Südbrasilens. *Stettiner Entomologische Zeitung* **73**, 16–45 (1938).
48. Brunetti, E. Revision of the Oriental Tipulidae with descriptions of new species. Part II. *Rec. Indian Mus.* **15**, 255–344 (1918).
49. Alexander, C. P. Diptera. Fam. Tipulidae. Subfam. Cylindrotominae. *Genera Insectorum.* **187**(1927), 1–16 (1928).
50. Meigen, J. W. Systematische Beschreibung der bekannten europäischen zweiflügeligen Insecten. I. *Friedrich Wilhelm Farstmann, Aachen*, i–xxxvi+1–332+[1] (1818).
51. Kopeć, K., Krzemiński, W., Skowron, K. & Coram, R. The genera *Architipula* Handlirsch, 1906 and *Grimmenia* Krzemiński & Zessin, 1990 (Diptera: Limoniidae) from the Lower Jurassic of England. *Palaeontol. Electron.* **20.1.15A**, 1–7 (2017).
52. Self, J. G., Johnson, R. C., Brownfield, M. E. & Mercier, T. J. Stratigraphic cross sections of the Eocene Green River Formation in the Piceance Basin, northwestern Colorado: US. *Geol. Surv. Digit. Data Ser. DDS-69-Y* **5**, 7 (2010).
53. Goloboff, P. A., Farris, J. & Nixon, K. TNT, a free program for phylogenetic analysis. *Cladistics* **24**, 774–786 (2008).
54. Goloboff, P. A. & Catalano, S. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* **32**, 221–238 (2016).
55. Geinitz, F. E. Über die Fauna des Dobbertiner Lias. *Zeitschrift der Deutschen Geologischen Gesellschaft* **36**, 566–583 (1884).
56. Handlirsch, A. Die fossilen Insecten und die Phylogenie der rezent Formen. Engelmann, Leipzig, (1906–1908).
57. Maddison, W. P. & Maddison, D. R. Mesquite: a modular system for evolutionary analysis. Version 3.61. <http://mesquiteproject.org>. Accessed 22 June 2020 (2009).
58. Nixon, K. C. WinClada ver. 1.00.08 Published by the author, Ithaca, New York (2002).
59. Nixon, K. C. ASADO, version 1.85 TNT-MrBayes Slaver (v1.5.30). Published by the author, Ithaca, New York (2004).

Acknowledgements

We would like to acknowledge for financial support of these research by JM Rector of University of Rzeszów (Al. Rejtana 16c, 35-601 Rzeszów). We would like to thanks two anonymous Reviewers for valuable comments and remarks.

Author contributions

I.K.-K. conceived and designed the study, lead and performed the data analysis, interpretations and writing, making photographs, graphical figures, analysis; correspondence. A.N. writing and corrections of the manuscript. J.S. corrections of phylogenetic analysis methods. W.J.-S. making photographs and drawing, graphical figures, analysis. W.K. corrections of the manuscript.

Funding

Open Access funding enabled and organized by the University of Rzeszów.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-021-91719-w>.

Correspondence and requests for materials should be addressed to I.K.-K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021

PHYLOGENY AND BIOGEOGRAPHY OF THE ENIGMATIC GHOST LINEAGE CYLINDROTOMIDAE (DIPTERA, NEMATOCERA)

Iwona Kania-Kłosok^{1*}, André Nel², Jacek Szwedo³, Wiktoria Jordan-Stasiło⁴, Wiesław Krzemiński⁵

Supplementary information

Supplementary Data S1

Cylindrotomidae is represented by nine extant genera: *Cylindrotoma*, *Diogma*, *Liogma*, *Phalacrocera*, *Stibadocera*, *Stibadocerella*, *Stibadocerina*, *Stibadocerodes*^[49], and *Triogma*. The Stibadocerinae comprise the four genera *Stibadocerodes*, *Stibadocera*, *Stibadocerella*, and *Stibadocerina*. The Cylindrotominae contain the genera *Cylindrotoma*, *Cyttaromyia*, *Diogma*, *Liogma*, *Phalacrocera*, and *Triogma*.

Supplementary Data S2

Cyttaromyia obdurescens Cockerell, 1925^[27]

(Fig. 1)

Redescription. Head (Fig. 1A, C) small, not well-preserved, eyes well-developed, large, occiput dark brown colored. Thorax brown (Fig. 1A) only approximately 1.5x as long as head, pronotum dark-brown. Sc (Fig. 1B, E, F) terminating in C well beyond level of fork of Rs, opposite approximately half length of R_{2+3+4} ; crossvein sc-r approximately one of its length from tip of Sc; R_{2+3+4} only shorter than d-cell and d'-cell; R_{3+4} longer than d-cell and d'-cell; section of R_5 from r'-m' connection to margin of wing shorter than d'-cell about 0.2x of length of this section; R_5 evenly curved towards margin wing; crossvein r-m positioned d-cell slightly distad of fork of Rs; four medial branches of Mb present; d-cell elongate, gradually widening towards wing margin, d-cell longer than apical sections of $M_1 - M_4$; d-cell set noticeably more basad than supernumerary cell (d'-cell), approximately 3.5x as long as wide; d'-cell noticeably narrows basad, approximately 5.5x as long as wide; crossvein m-cu located beyond fork of Mb on M_{1+2} and M_{3+4} ; base of apical part of M_1 almost perpendicular to M_2 , before turning towards apex of wing; M_1 (excluding base of M_1 , before turning towards apex of wing) and M_2 almost equal in length, slightly shorter than M_3 and slightly longer than M_4 ; apical section of M_3 subsinuous from d-cell towards wing margin; distance between tip of R_5 and tip of M_1 or tip of M_1 and tip of M_2 almost equal in length; distance between tip of M_3 and M_4 2x as long as distance between tip of M_2 and M_3 ; distances between tip of M_2 and M_4

and between tips of M_4 and Cu almost equal in lengths. A_2 elongate, slightly sinusoidal. Tip of A_1 positioned behind fork of Rs. Haltera (Fig. 1D) elongate, shorter than 0.25x of fore wing. Abdomen (Fig. 1G) approximately 5x as long as thorax, ovipositor not very elongate.

***Cyttaromyia gelhausi* sp. nov.**

<http://zoobank.org/urn:lsid:zoobank.org:act:9165E3D2-514B-4524-9F85-A9895CBF2A31>

(Figs 2 – 3)

Description. Body length 6.24 – 6.83 mm (male) (Fig. 2A), 6.72 mm (female) (Fig. 2D), wing length 7 mm (male) (Fig. 2A, 3A-C), 6.89 mm (female) (Fig. 2D). Head 1.10 – 1.93 mm wide (male) (Fig. 2A, B), 1.5 mm (female), small, not well-preserved, compound eyes well-developed, large; occiput dark brown colored; last segment of antennae rather elongate, slightly longer than penultimate one; antenna poorly preserved; palpi not visible.

Thorax brown (Fig. 2A) approximately 2.5x as long as head, pronotum dark-brown. Wing length 6.59 mm, width 1.48 mm (male), 6.35 – 7.47 mm, width 1.50 – 1.70 mm (female) with a pronounced stigma in distal part of wing; Sc (Fig. 3A-C) terminating in C just beyond level of fork of Rs, opposite approximately 1/10 length of R_{2+3+4} ; position of crossvein sc-r approximately one of its length from tip of Sc; R_{2+3+4} shorter than d-cell and d'-cell; R_{3+4} longer than d-cell and d'-cell; section of R_5 from r'-m' connection to margin of wing shorter than d'-cell about 0.25x length of this section; R_5 evenly curved towards margin wing; crossvein r-m situated slightly distad of fork of Rs; four medial branches of Mb presented; d-cell elongate, gradually widening towards wing margin, d-cell longer than apical sections of $M_1 - M_4$; d-cell set noticeably more basad than supernumerary cell (d'-cell), approximately 2.5x as long as wide; d'-cell noticeably narrows basad, approximately 3.5x as long as wide; crossvein m-cu located at fork of Mb on M_{1+2} and M_{3+4} ; base of apical part of M_1 almost perpendicular to M_2 , before turning towards apex of wing; M_1 (excluding base of M_1 , before turning towards apex of wing) and M_2 almost equal in length, slightly shorter than M_3 and slightly longer than M_4 ; apical section of M_3 subsinuuous from d-cell towards wing margin; distance between tip of R_5 and tip of M_1 or tip of M_1 and tip of M_2 almost equal in length; distance between tip of M_3 and M_4 2x as long as distance between tip of M_2 and M_3 ; distance between tip of M_2 and M_4 longer than distance between tips of M_4 and Cu. Haltera elongate, shorter than 0.25x of wing.

Abdomen (Fig. 2A, C, D) approximately 2.5x as long as thorax, ovipositor not very elongate, 1.37 mm long, about 0.3x length of abdomen; hypopygium 1.09 mm long, wide, rather short, gonocoxites wide, massive.

***Cyttaromyia freiwaldi* sp. nov.**

(Figs 4 – 5)

<http://zoobank.org/urn:lsid:zoobank.org:act:DB604450-AD47-4644-82E0-A33FEAEB7157>

Description. Body (Figs 4B; 5A, B) length 4.8 mm, wing length 6.14 – 6.22 mm (Figs 4B, C, D; 5 A, B, E, F). Head (Figs 4A, B; 5C) 1.33 mm wide, compound eyes well-developed, large, occiput dark brown colored; antenna rather short, not very well preserved, 2.32 – 2.36 mm; palpi not well visible.

Thorax brown (Figs 4B; 5A, B) approximately 2.5x as long as head, pronotum brown. Wing length 6.14 – 6.22 mm, with a pronounced stigma in distal part of wing; Sc (Fig. 4C, D) terminating in C just beyond level of fork of Rs, opposite approximately 1/10 the length of R_{2+3+4} ; crossvein sc-r approximately one of its length from tip of Sc; R_{2+3+4} shorter than d-cell and d'-cell; R_{3+4} longer than d-cell and d'-cell; section of R_5 from r'-m' connection to margin of wing shorter than d'-cell about 0.2x length of this section; R_5 evenly curved towards margin wing; crossvein r-m positioned slightly distad of fork of Rs; four medial branches of Mb presented; d-cell elongate, gradually widening towards wing margin, d-cell longer than apical sections of $M_1 - M_4$; d-cell set noticeably more basad than supernumerary cel (d'-cell), approximately 3.5x as long as wide; d'-cell only slightly narrows basad, approximately 3.5x as long as wide; crossvein m-cu located beyond fork of Mb on M_{1+2} and M_{3+4} in 0.2 length of M_{3+4} ; base of apical part of M_1 almost perpendicular to M_2 , before turning towards apex of wing; M_1 (excluding base of M_1 , before turning towards apex of wing) and M_2 almost equal in length, longer than M_3 and M_4 ; apical section of M_3 almost straight from d-cell towards wing margin; distances between tip of R_5 and tip of M_1 or tip of M_1 and tip of M_2 almost equal in length; distance between tip of M_2 and M_3 1.5x as long as distance between tip of M_3 and M_4 ; distance between tip of M_2 and M_4 longer than distance between tips of M_4 and Cu. Haltera elongate, shorter than 0.2x of wing.

Abdomen (Figs 4B; 5A, B, D) approximately 5x as long as thorax, ovipositor not very long, 2.4 mm long, about 0.25x length of abdomen.

Supplementary Data S3

Characters scored for phylogenetic analysis.

1. Proportion between length of antenna and length of body: antennae one-third body length or shorter (0); antennae comparatively long, longer than one-third body length (1).

2. Shape of flagellomeres: not serrate (0); serrate (1).
3. Proportion between length and width of first and second flagellomeres: less than 3x longer than wide (0); more than 3x longer than wide (1).*
4. Coloration of wing: hyaline (0); at least partially pigmented brownish (1).
5. Length of vein Sc: Sc rather elongate, terminate opposite fork of Rs level or beyond (0); Sc short, terminate before fork of Rs level (1).
6. Tip of Sc: Sc reaching wing margin (0); Sc partially atrophied, not reaching wing margin (1); tip of Sc completely atrophied, not reaching wing margin (2).
7. Crossvein sc-r: well-developed (0); tendency to atrophy (1).
8. Branches of radial vein Rb: four branches merging into wing apex (0); three branches merging into wing apex (1); two branches merging into wing apex (2).
9. Number of branches of Rs reaching wing margin: at least two (0); one (1).
10. Length of vein R₁: R₁ terminating beyond level of fork of M₄ and M₃ (0); R₁ terminating at level of fork of M₄ and M₃ (1).
11. Vein R₅: well-developed (0); atrophied (1).
12. Shape of base of R₂₊₃₊₄: slightly waved (0); strongly curved (1).
13. Position of R₅: R₅ separated from point of connection of r-m and M₁₊₂ (0); R₅ separated from margin of d-cell (1).
14. Relationship of M₁ and M₂: at least partially separate (0); fused (1).
15. Presence of petiole: absent (0); present (1).
16. A supernumerary crossvein r'-m' connected vein R₅ with M₁ near its origin, to produce two discal cells: absent (0); present (1).
17. R₃ and R₄: separate (0); fused (1).
18. Position of crossvein m-cu relative to bifurcation of M₃₊₄: m-cu located at fork of M₃₊₄ (0); m-cu located at extreme base of discal cell or before it (1).
19. Position of crossvein m-cu relative to bifurcation of Mb: beyond fork of Mb (0); at fork of Mb (1).
20. Shape of d-cell: rhomboid (0); rectangular (1).
21. Position of medial veins: M₁₊₂ or M₂ and M₃ separated (0); M₁₊₂ and M₃ having at least a point of contact at base (1).
22. Shape of base of M₃: not indented (0); strongly indented (1).
23. The distance between tip of M₄ and tip of Cu: narrow (0); very wide (1).
24. Crossvein r-m: well-developed (0); reduced (1).
25. Position of A₂ tip: beyond Rs origin level (0); before of Rs origin level (1).

26. Position of A₁ tip: at or beyond of d-cell level (0); before of d-cell level (1).
27. Gonostylus: two-branched with well-developed clasper and lobe (0); two-branched, but with reduced lobe (1); with a single branch (2).*
28. Aedeagus: single (0); bifid (1); trifid (2).*

Supplementary tables

Supplementary Table S1. List of fossil species of *Cyttaromyia* (partially after used information from [26, 40]).

SPECIES	AGE (MA)*	TYPE HORIZON
<i>Cyttaromyia fenestrata</i>	37.2 – 33.9	USA/Green River Formation
<i>Cyttaromyia frelloi</i>	38.0 – 33.9	Baltic amber
<i>Cyttaromyia fuscula</i>	37.2 – 33.9	USA/Green River Formation
<i>Cyttaromyia lynnae</i>	46.6 – 45.8	USA/Kishenehn Formation
<i>Cyttaromyia obdurescens</i>	50.3 – 46.2	USA/Green River Formation/Roan Moutains/Colorado
<i>Cyttaromyia princetoniana</i>	37.2 – 33.9	USA/Florissant Formation
<i>Cyttaromyia quievreuxi</i>	33.9 – 28.1	France/Middle Salt Formation
<i>Cyttaromyia rayona</i>	38.0 – 33.9	Russia/Biamo Formation
<i>Cyttaromyia reclusa</i>	50.3 – 46.2	USA/Green River Formation/Roan Moutains/Colorado
<i>Cyttaromyia rossi</i>	38.0 – 33.9	UK/Isle of Wight/Bembridge Marls
<i>Cyttaromyia scudderi</i>	56.0 – 47.8	Denmark/Ølst Formation
<i>Cyttaromyia vahldieki</i>	56.0 – 47.8	Denmark/Ølst Formation

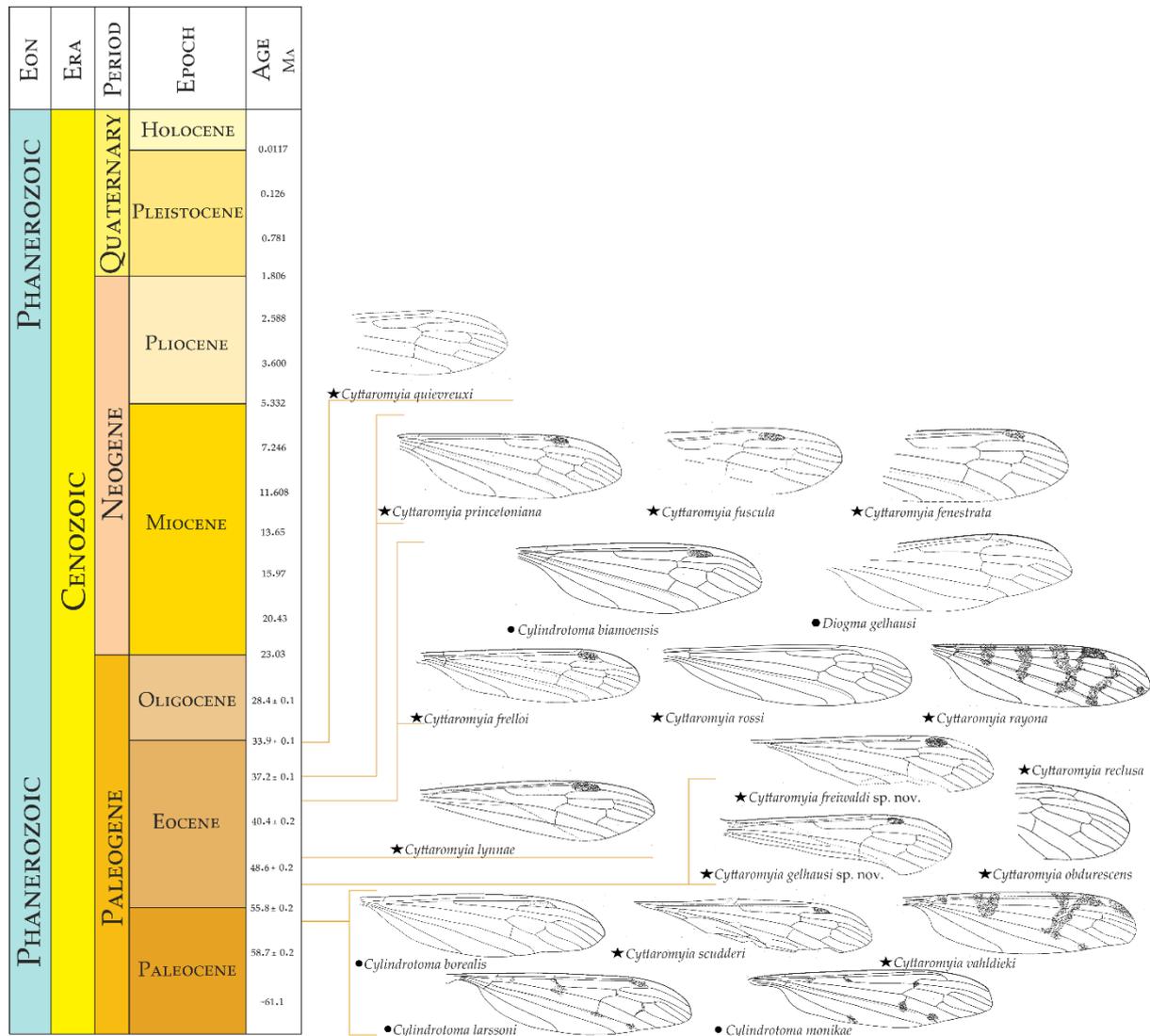
* Ages are given after [6, 41]

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
<i>Architipula seebachi</i>	1	0	0	0	0	0	0	0	0	0	1	1	?	?
<i>Cylindrotoma distinctissima distinctissima</i>	0	0	1	1	0	1	0	0	0	0	1	0	2	2
<i>Cyttaromyia fenestrata</i>	0	1	1	1	0	1	0	0	0	0	0	1	?	?
<i>Diogma glabrata</i>	0	0	1	1	0	1	0	0	0	0	0	0	0	1
<i>Liogma nodicornis</i>	0	0	1	1	1	1	0	0	0	1	0	1	2	2
<i>Phalacrocerera replicata</i>	0	0	1	1	1	1	1	0	0	0	0	0	2	2
<i>Triogma trisulcata</i>	0	0	1	1	1	1	0	0	0	1	0	0	2	2
<i>Tipula oleracea</i>	1	0	0	0	0	0	0	0	0	0	1	1	0	0
<i>Stibadocera bullans</i>	0	0	2	1	0	1	0	1	1	0	0	0	1	2
<i>Stibadocerella pristina</i>	0	0	2	1	0	1	0	1	1	0	0	1	0	2
<i>Stibadocerina chilensis</i>	0	0	2	1	0	1	0	1	1	0	0	1	2	2
<i>Stibadocerodes australiensis</i>	0	0	1	1	0	1	0	1	1	0	0	0	0	2

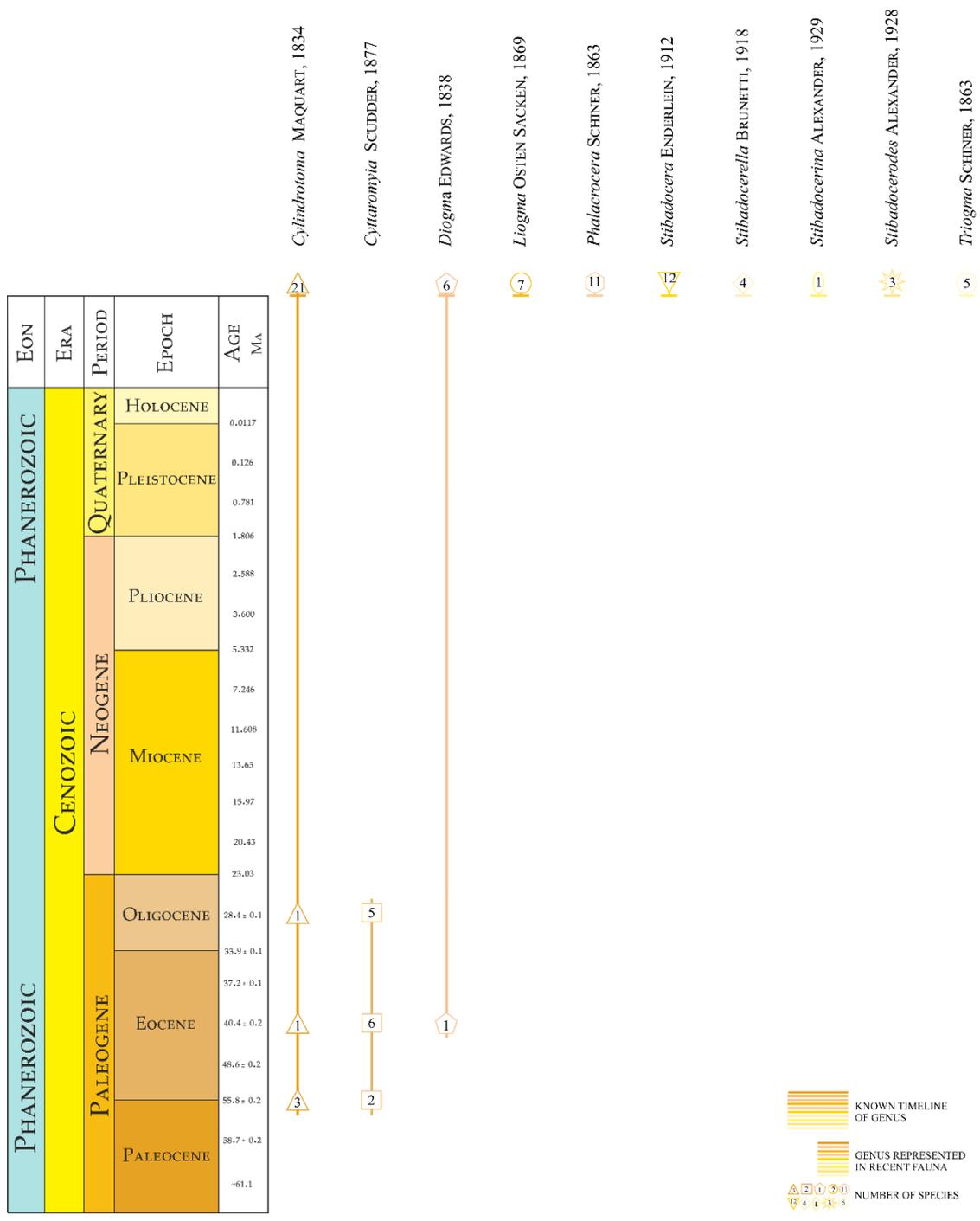
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Architipula seebachi</i>	?	?	?	0	0	0	0	0	0	0	0	0	0	0
<i>Cylindrotoma distinctissima distinctissima</i>	0	0	0	0	0	1	1	2	0	0	0	0	0	0
<i>Cyttaromyia fenestrata</i>	?	0	?	0	0	0	0	2	0	0	0	0	0	0
<i>Diogma glabrata</i>	0	0	0	0	0	2	0	2	0	0	0	1	0	1
<i>Liogma nodicornis</i>	0	1	0	0	1	0	0	2	0	1	0	1	1	1
<i>Phalacrocerera replicata</i>	0	0	0	1	1	2	0	1	0	0	0	0	0	1
<i>Triogma trisulcata</i>	0	1	0	0	0	1	1	1	0	1	0	1	1	1
<i>Tipula oleracea</i>	0	0	1	1	1	2	0	0	0	0	0	0	0	0
<i>Stibadocera bullans</i>	1	0	1	0	1	2	0	1	1	1	1	0	0	1
<i>Stibadocerella pristina</i>	1	0	1	0	1	0	0	2	1	0	1	0	0	1
<i>Stibadocerina chilensis</i>	1	0	1	0	1	0	0	2	1	0	1	0	0	1
<i>Stibadocerodes australiensis</i>	1	0	1	0	1	0	0	2	0	0	1	0	0	1

Supplementary Table S2. Matrix of characters

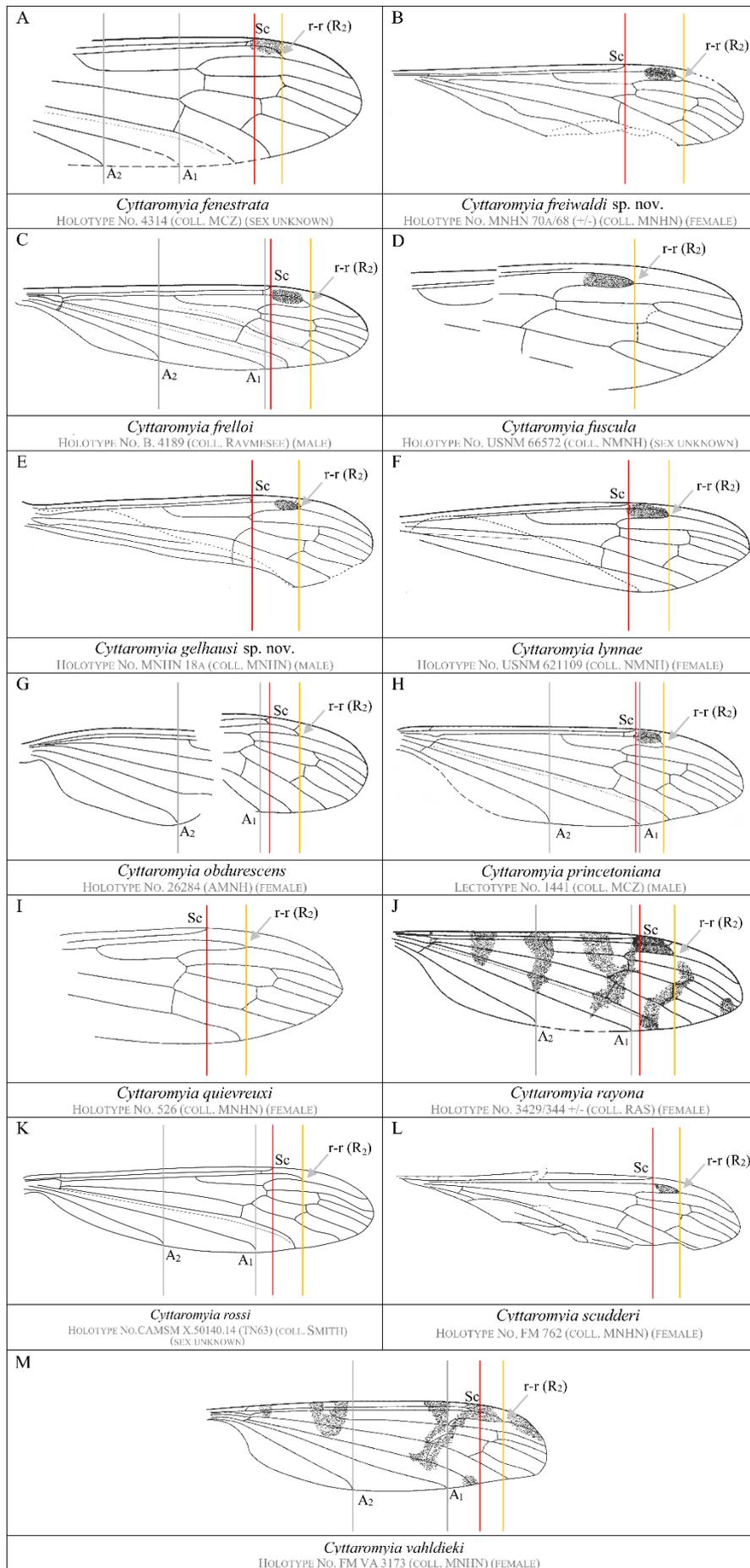
Supplementary figures



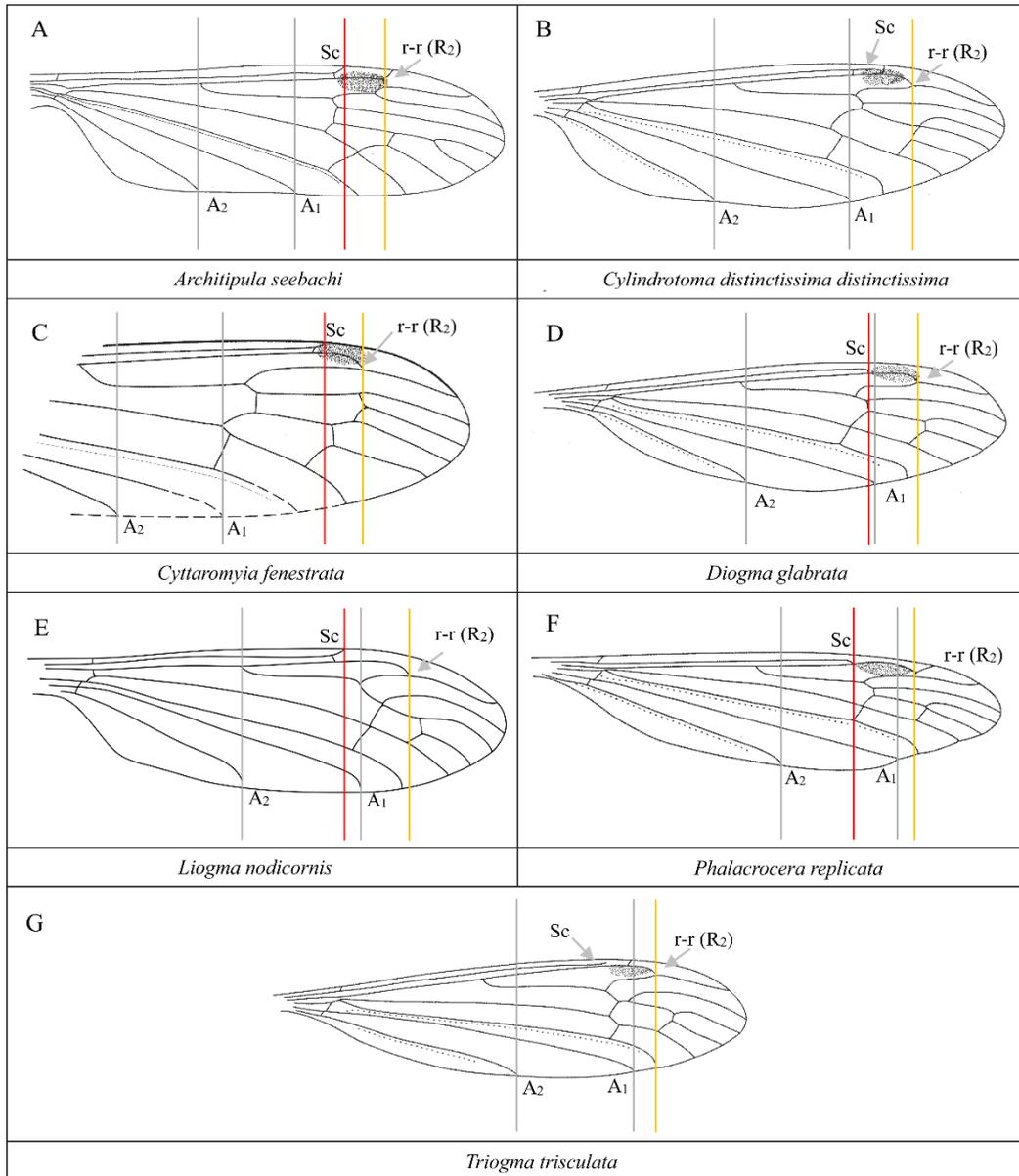
Supplementary Figure S1. Wing venation and color pattern of fossil Cyldrotominae with chronostratigraphic distribution view, wing venation redrawn [24, 25, 27-29, 31, 34, 40, 48]. Stratigraphic chart according to International Stratigraphic Chart, International Commission of Stratigraphy (v. 2021/05) <https://stratigraphy.org/chart>.



Supplementary Figure S2. Chronostratigraphic distribution of Cyliodontomidae. In white figures the number of species is given. Lines mark time of duration of the genus. Stratigraphic chart according to International Stratigraphic Chart, International Commission of Stratigraphy (v. 2021/05) <https://stratigraphy.org/chart>.



Supplementary Figure S3. Wing venation of species of *Cyttaromyia*, redrawn^[28-31, 39, 40]. Abbreviations: lines cut the points: grey line – tips of A₁ or A₂; red line – tip of Sc; yellow line – to tip of r-r (R₂).



Supplementary Figure S4. Wing venation of type species of Cylindrotominae and the genus *Architipula* – as an outgroup used in parsimony analysis, redrawn^[39,49, 60]. Abbreviations: lines cut the points: grey line – tips of A₁ or A₂; red line – tip of Sc; yellow line – to tip of r-r (R₂).

Supplementary references

60. Alexander, C.P. Diptera of Patagonia and South Chile. Part I. Crane-flies (Tipulidae, Trichoceridae, Tanyderidae). *Diptera of Patagonia and South Chile*, **1**, 1-240 (1929).