



Cretaceous Horse flies and their phylogenetic significance (Diptera: Tabanidae)

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Abstract

Popularly known as horse flies or deer flies, Tabanidae, has 4.400 described species distributed worldwide. Most of the females are hematophagous, but several species are also flower visitors. Cretaceous fossils of Tabanidae are scarce and the known fossil species have plesiomorphic features unknown in modern horse flies. Here, we revised the taxonomy of the Tabanidae from the Crato Formation of Brazil describing a new genus, *Araripus* **gen. nov.**, and two new species – *Araripus crassitibialis* **sp. nov.** and *Cratotabanus cearensis* **sp. nov.** The holotype of *Cratotabanus stonemyomorphus*, the type species of *Cratotabanus*, is redescribed, and the male is described for the first time. In addition, we investigate the phylogenetic position of Cretaceous horse fly fossils using morphological characters in the context of a wider analysis also including representatives of extant lineages.

Keywords

bayesian inference, fossils, morphology, parsimony, Tabanidae, terminalia

1. Introduction

With nearly 4.400 described species distributed in all biogeographic regions, Tabanidae constitutes one of the largest families of the order Diptera (Evenhuis and Pape 2022) and by far the richest family within the infraorder Tabanomorpha in number of species. Horse flies are known for their medium to large size (5 to 25 mm) and painful bites – most females are hematophagous, using blood to nourish their eggs. Males and females of some

species, are also flower visitors, and species of at least one genus, *Philoliche* Wiedemann, are known as pollinators (Johnson and Morita 2006).

Traditionally, Tabanidae is divided into four subfamilies, based mostly on genital characters: Pangoniinae, Scepsidinae, Chrysopsinae, and Tabaninae (Mackerras 1954). Recent phylogenetic hypotheses have challenged this classification, and some of the morphologically rec-

ognized subfamilies and tribes may not constitute monophyletic groups (Morita et al. 2016).

The horse flies fossil record is relatively abundant, dating back to the Early Cretaceous (see Strelow et al. 2013 for a summary). Most fossils are from the Tertiary; Mesozoic fossils are scarce and usually represented by compressions (Evenhuis 1994; Martins-Neto 2003; Grimaldi 2016). Early Tabanidae fossils have little resemblance with modern known taxa and no Cretaceous specimens could be safely identified as representative of any extant horse fly subfamily.

The first horse fly fossil species described is *Cratotabanus stonemyomorphus* Martins-Neto and Santos, from the Lower Cretaceous (Aptian) Crato Formation, based on a single female specimen (Martins-Neto and Santos 1994). More recently, other species of *Cratotabanus* were described from Upper Cretaceous (Turonian) amber from New Jersey (Grimaldi et al. 2011) and Mid-Cretaceous Burmese amber (Grimaldi 2016). One of the *Cratotabanus* species from the Burmese amber, *Cratotabanus asiaticus*, has a two-segmented cercus, a character absent in modern Tabanidae, and an undivided tergite X, a character believed to be autapomorphic for three enigmatic extant genera (Philip and Coscarón 1971). This may indicate that *Cratotabanus* belongs to a stem lineage of Tabanidae.

Tabanipriscus transitivus Grimaldi, also from Burmese Amber, is positioned within Tabanidae, and several plesiomorphic characters suggests it to be a stem group Tabanidae or a stem-group Athericidae + Tabanidae (Grimaldi 2016). Other Cretaceous fossils include *Eopangonius pletus* Ren (Yixian Formation, Lower Cretaceous of China), *Baissomyia redita* Mostovsky, Jarzembowski and Coram (Lower Cretaceous, Zaza Formation of Russia), *Eotabanoid lord* Mostovsky, Jarzembowski and Coram (Lower Cretaceous, Purbeck Group of England) and *Laiyangitabanus formosus* Zhang (Lower Cretaceous, Laiyang Formation of China). *Palaepangonius* Ren, the only Cretaceous fossil originally attributed to an extant tabanid subfamily, was described as a Pangoniinae but then was placed as incertae sedis within Tabanomorpha (Grimaldi 2016).

According to molecular divergence time estimations, Pangoniinae – the oldest modern horse fly subfamily – originated between the mid-Cretaceous (100 MY) and the Early Paleogene (45 MY) (Morita et al. 2016). The molecular data and the absence of putative fossils from modern subfamilies have led some to hypothesize that early Tabanidae forms went extinct, while modern lineages diversified between the end of the Cretaceous and the beginning of the Paleogene, driven by the dominance of Angiosperms and the Mammalian radiation (Labandeira 2010; Morita et al. 2016).

The goals of the present paper are twofold. First, we present a taxonomic revision of the horse fly fossils from Brazilian Crato Formation, with the description of new taxa. In addition, we investigate the phylogenetic position of Cretaceous horse fly fossils using morphological characters, in the context of a wider analysis also including representatives of extant lineages.

2. Material and Methods

2.1. Geological context of the new taxa

All the new fossil taxa of horse flies described here are from the Lower Cretaceous Crato Formation of Brazil. The geology and paleontology of the Crato Formation, an important Gondwanan Konservat-Lagerstätten, was revised in detail by Martill et al. (2007) and Ribeiro et al. (2021). The Crato Formation is one of the stratigraphic units that constitute the Santana Group of the Araripe Basin. From the base to the top, the Santana Group consists of the Barbalha, Crato, Ipubi, and Romualdo formations. The age of the Crato Formation is considered to be upper Aptian (Lower Cretaceous) (Heimhofer and Hochuli 2010).

According to Ribeiro et al. (2021), the fossil-rich interval of the formation – the “Crato Konservat-Lagerstätte” or CKL –, consisted of a semi-arid seasonal lacustrine wetland, in which a shallow water body, including diverse aquatic fauna and flora, was succeeded up-landward by neighboring mesophytic ecotones, periodically flooded, besides outer xeric habitats. Trophic structure analysis and details of the putative food web that took place within the Crato Ecosystem are provided by Mendes et al. (2020).

2.2. Material and terminology

The terminology used in the morphological study follows the interpretation of Cumming and Wood (2017), and Chainey (2017). The studied specimens are housed in the collections of the Instituto de Geociências da Universidade São Paulo (IGC-USP, São Paulo, Brazil) and the Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (CCNH-UFABC, Santo André, Brazil).

All specimens were photographed using a Motic K700-L stereo microscope with a canon EoS Rebel T2i digital camera attached, and photos were edited using GIMP 2.10 software. Drawings were made in Inkscape 1.1. Measures and scales were made with Carl Zeiss AxioVision (Release 4.8) software.

2.3. Phylogenetic analysis

The ingroup sampling included 20 extant horse flies from three of the traditionally recognized subfamilies (Pangoniinae, Chrysopsinae, Tabaninae). Sepsidinae was not included since it has only two very dissimilar species, and it is not recognized by some authors (e.g. Coscarón and Papavero 2009b). All species described for *Cratotabanus* Martins-Neto and Santos and *Araripus* **gen. nov.** were included as terminals in our analysis. *Tabanipriscus transitivus* Grimaldi was also included since the species has diagnostic traits of Tabanidae and also traits of the Tabanomorpha groundplan such as a two-segmented cer-

cus and the absence of a postspiracular scale (Grimaldi 2016), which are useful to understand the early relationships of Tabanidae.

Our morphological matrix is based on previous literature (Mackerras 1954; Stuckenberg 1973; Yeates 2002; Kerr 2010; Grimaldi et al. 2011; Grimaldi 2016) and new observations. The outgroup is composed of two extant species of Rhagionidae – *Rhagio mystaceus* (Maquart) and *Atherimorpha lamasi* Santos – and three species of Athericidae – *Suragina pacaraima* Rafael and Henriques, *Atherix ibis* (Fabricius) and *Dasyomma caeruleum* Macquart. It includes 35 morphological characters and 31 terminal taxa, with 25 extant and 6 fossil taxa. The chosen outgroups and the codified characters are relevant to the understanding of family and subfamily phylogenetic positioning. The matrix was analyzed through parsimony and Bayesian Inference. Then, the final topologies for each of the optimality criteria were compared.

For the parsimony analysis, trees were rooted between the Rhagionidae and the Athericidae + Tabanidae, on the earliest divergence of the outgroups following Nixon and Carpenter (1993). The analysis was performed in TNT (version 1.5; Goloboff and Catalano 2016) using both equal weights and implied weighting (Goloboff 1993, 2014; Goloboff et al. 2008). For the implied weighting analyses, we used k values of 5, 15 and 150. All analyses were performed using heuristic searches with TBR branch swapping, random stepwise addition sequence, and 5000 replicates holding up to 10 trees per replication, keeping the maximum possible number of trees in memory (99999 trees).

Bayesian analyses were performed on MRBAYES 3.2.7 (Ronquist et al. 2012) under the MK model (Lewis 2001) with a lognormal distribution for modeling character rate variation. The analysis was carried out with two simultaneous runs, each with four chains. Each run contained 10 million generations of the mcmc chain, with trees sampled every 1000 generations and a burning cut-off set at 25 percent. Convergence was checked by the standard deviation of the sample spits and by examining trace plots using TRACER 1.7 (Rambaut et al. 2018).

A complete list of the taxa and the data matrix used in the phylogenetic analyses are provided in the Supplementary material.

3. Results

3.1. Systematic paleontology

Cratotabanus Martins-Neto and Santos

Figs 1–4

Cratotabanus Martins-Neto and Santos 1994: 291, fig. 1, pl. 1; Grimaldi et al. 2011: 303 (revised diagnosis); Grimaldi 2016: 45, fig. 16A (emended diagnosis).

Diagnosis. Ocellar triangle developed with visible ocelli; basal callus absent; postspiracular scale absent; R_5 slightly curved; hind tibia with 2 spurs; tergite X undivided; cercus two-segmented.

Comments. The genus *Cratotabanus* differs from extant horse flies by the presence of two-segmented cercus and by the absence of the postspiracular scale, a one-segmented cercus and a postspiracular scale is shared by all extant Tabanidae (Mackerras 1954). Grimaldi (2016) also pointed out that the length of the discal cell in *Cratotabanus* is nearly the same as the length of the vein M_1 . However, this character is variable in the extant species. The genus can be attributed to Tabanidae by the general disposition of the wing veins, the general shape of the body, the clypeus format and the presence of an enlarged calypter (Grimaldi 2016, and our Figs 1, 2).

Cratotabanus differs from *Laiyangitabanus* Zhang and *Eotabanoid* Mostovski et al. by the presence of vein R_{2+3} curved only at the apex. From *Laiyangitabanus*, *Cratotabanus* further differs by the presence of a well-developed ocellar triangle, and r-m closer to the base of the discal cell (Grimaldi 2016). *Cratotabanus* differs from *Eopangonius* Ren by the absence of basal callus and a not projected clypeus.

Cratotabanus stonemyomorphus Martins-Neto and Santos

Figs 1, 2

Cratotabanus stonemyomorphus Martins-Neto and Santos, 1994: 291, fig. 1, pl. 1; Morita et al. 2016 (Tabanidae phylogeny).

Cratotabanus stonemyomorphus Coscarón and Papavero, 2009b (Cat., as unrecognized, error)

Cratotabanus stonemyomorphus Martins-Neto 2003: 31, fig. 2 (Fossil tabanids, error); Bechly 2007: 386 (Crato insects spp, error); Grimaldi et al. 2011: 303 (Cretaceous amber spp., error); Zhang 2012: 1 (Lower Cretaceous China, error); Strelow et al. 2013: 439 (summarized Tabanidae fossil record, error); Grimaldi 2016: 45, fig. 1A (Cretaceous Myanmar spp., error).

Examined material. *Holotype female*: GP1T/2585. NE Brazil, Crato Formation, Aptian, Lower Cretaceous. — Male, CCNH 141, NE Brazil, Crato Formation, Aptian, Lower Cretaceous

Preservation. Preserved in ventral view. Clypeus, scape and pedicel visible. Meso and meta femurs, hind left leg, wings, and abdomen preserved.

Diagnosis. Frons apparently parallel or slightly convergent; R_1 apex inserting in C at the same level of discal cell; vein R_4 slightly curved.

Description. Female. Measurements: Moderate size, body 9.3 mm, wing 6.9 mm. — **Head:** Frons apparently parallel or convergent; basal callus absent; subcallus not protuberant or greatly developed; scape and pedicel with similar size; clypeus not conical or projected. — **Wings:**

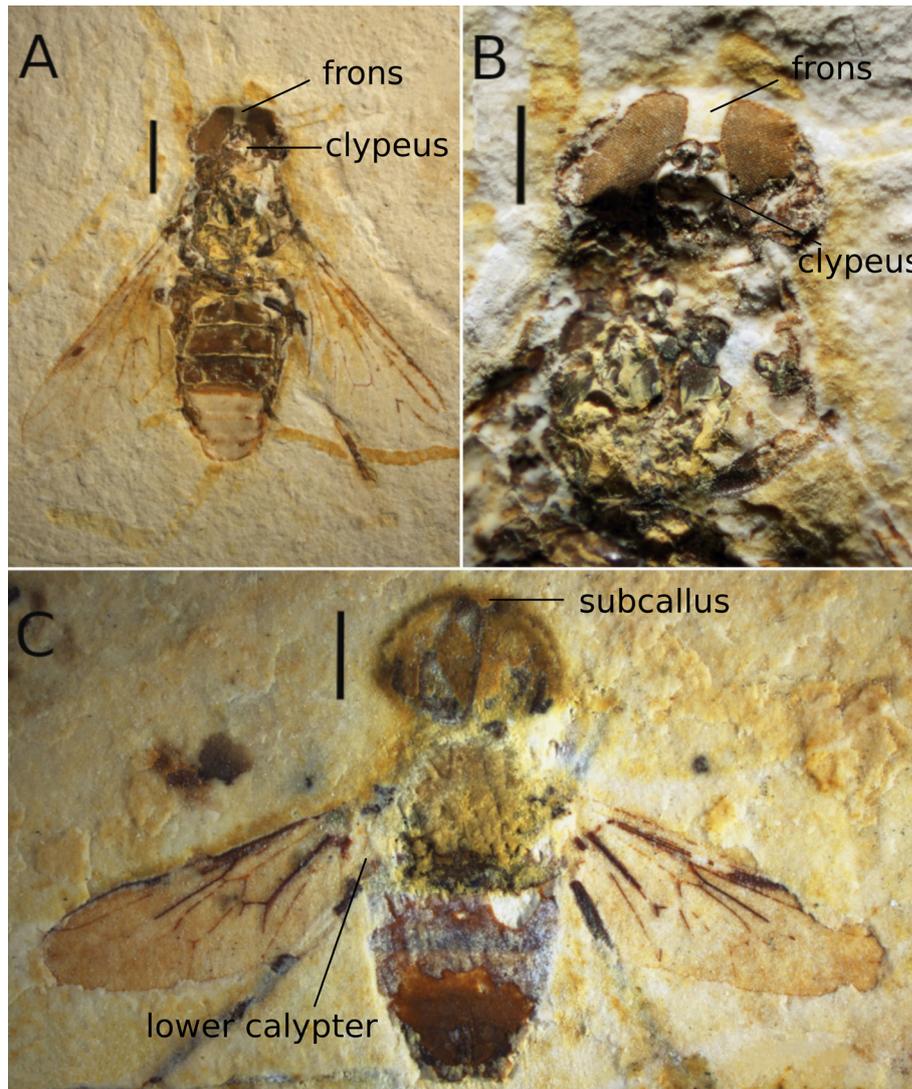


Figure 1. *Cratotabanus stoneomyomorphus*. Martins-Neto and Santos. **A, B** Holotype female. **A** habitus preserved in ventral view. **B** Detail of head. **C** Male, preserved in dorsal view. Scale bars: 2 mm (A, C); 1 mm (B).

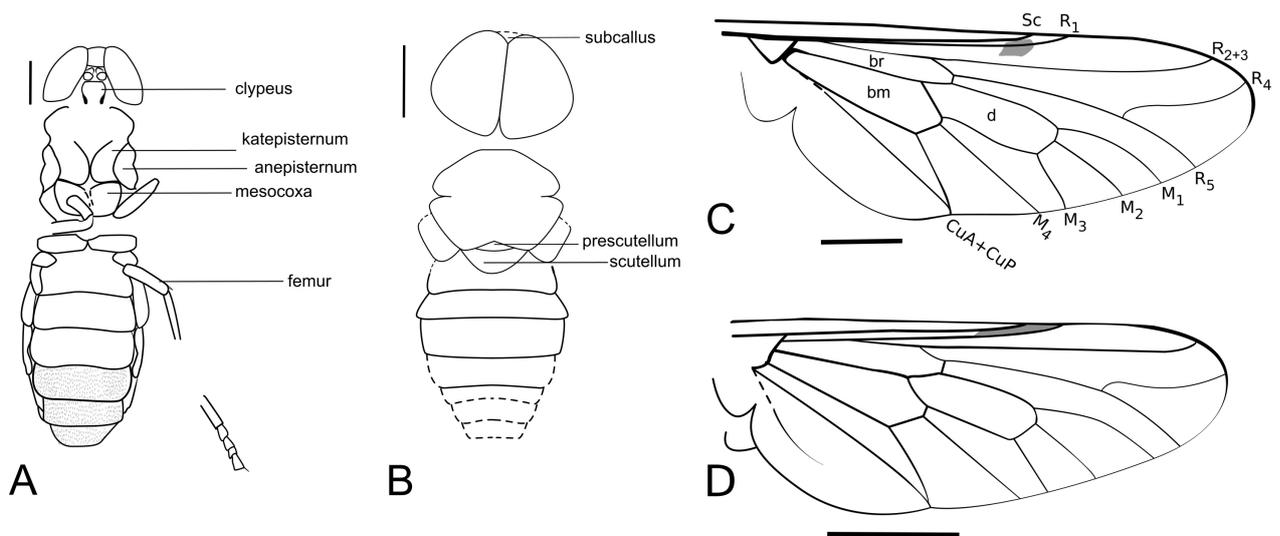


Figure 2. *Cratotabanus stoneomyomorphus*. **A** Holotype female, illustration of dorsal habitus. **B** Male, illustration of ventral habitus. **C** Holotype female, wing. **D** Male, Wing. Scale bars: 2 mm (A, C); 1 mm (C, D).

Wing *ca.* 3 times longer than wide; pterostigma small or partially preserved, close to the insertion of R_1 on C; apex of R_1 near the to same level of cell d apex; R_{2+3} almost

straight, curved at the insertion in C; vein R_4 slightly curved; no appendix at R_4 ; cell r_5 open; M_1 curved, parallel to M_2 ; M_3 divergent from M_2 ; cell m_3 open; m-m two

times longer than fork of M_1 and M_2 to the m-m insertion; m-cu three times longer than origin of M_4 to the m-cu insertion; CuA meeting CuP before wing margin; lower calypter pronounced. – **Abdomen:** Sternites V to VII with visible setae. — **Male. Measurements:** Moderate size, body 11.3 mm, wing 7.9 mm. – **Head:** Head hemispherical and holoptic, subcallus forming a prominent triangle; no visible differences among ommatidia size. – **Thorax:** scutum badly preserved, stout, nearly as wide as long, apparently lighter than abdomen; prescutellum present, scutellum and prescutellum darker than scutum, prescutellum badly preserved. – **Legs:** dark. – **Wings:** wing as in female, except by the apparently longer pterostigma. – **Abdomen:** abdomen with seven visible segments, seventh segment badly preserved.

Comments. The differences between *Cratotabanus stonemyomorphus* and other species of the genus were discussed by Grimaldi et al. (2011) and Grimaldi (2016). Here, we describe a male specimen that we believe to be *C. stonemyomorphus* given the nearly identical wing venation. The male adds important information about this species as the presence of an holoptic head – which is present in most extant Tabanidae –, the presence of the prescutellum and an enlarged lower calypter, considered synapomorphy of Tabanidae. The last two characters have already been observed in *C. asiaticus* by Grimaldi (2016) but not preserved in the female of *C. stonemyomorphus*.

Cratotabanus cearensis Carmo and Sampronha sp. nov.

<http://zoobank.org/25421A10-26E4-4075-984F-92C36F6F1431>

Figs 3, 4

Examined material. Holotype female: CCNH 567, NE Brazil, Crato Formation, Aptian, Lower Cretaceous.

Preservation. Preserved in lateral view. Frons and occiput visible. Notopleuron, anepisternum, katepisternum and scutellum preserved. Fore, mid and hind leg preserved. Wings partially preserved. Tergites of abdomen and female cercus preserved.

Diagnosis. Frons apparently convergent; apex of R_1 near to the same level of end of cell d; R_4 strongly curved at posterior half; vein m-cu two times longer than the origin of M_4 to the m-cu insertion; M_2 and M_3 sub-parallel; suture at sternite I+II strongly marked.

Description. Holotype female. Measurements: Moderate size, body 9.6 mm, wing 6.9 mm. – **Head:** Frons apparently convergent bellow; ocellar triangle developed with ocelli; basal callus absent. – **Legs:** hind leg with two tibial spurs; – **Wings:** Apex of R_1 near to the same level of cell d apex; R_{2+3} straight (insertion in C not visible);

vein R_4 strongly curved at posterior half; no appendix at R_4 ; cell r_5 open; M_1 curved, parallel to M_2 ; M_3 sub-parallel to M_2 ; cell m_3 open; m-m three times longer than fork of M_1 and M_2 to the m-m insertion; m-cu two times longer than origin of M_4 to the m-cu insertion; wing ca. 3 times longer than wide. – **Abdomen:** tergite X undivided; – **Terminalia:** Cercus two-segmented, anterior segment an inverted triangle, posterior segment ovoid.

Etymology. The name of the species refers to the state of Ceará (NE Brazil) where the Araripe Basin is located.

Comments. *Cratotabanus cearensis* sp. nov. has a two-segmented cercus, a character also visible in *C. asiaticus* Grimaldi (Grimaldi 2016) and *Araripus crassitibialis* gen. nov. et sp.nov., which follows the ‘bauplan’ of Tabanomorpha. Tergite X is visible in lateral view, apparently undivided as in *C. asiaticus*. *Cratotabanus cearensis* sp. nov. differs from *C. stonemyomorphus* by the vein m-cu two times longer than the joint between the discal cell and the base of M_4 (against three times in *C. stonemyomorphus*), and vein R_4 strongly curved at posterior half. From *C. newjerseyensis* Grimaldi, *C. cearensis* sp. nov. differs by the veins M_2 and M_3 almost parallels and m-m three times longer than the distance between the bases of M_1 and M_2 – in *C. newjerseyensis* they are nearly the same size (Grimaldi 2016). *C. cearensis* sp. nov. differs from *C. asiaticus* by having the apex of R_1 inserting in C near to the same level of cell d apex. The specimen here described is preserved in lateral view, with seven visible abdominal sternites. In all known extant and fossil Tabanidae, the sternites I and II are fused. In most species, however, a remnant suture of variable prominence in the resultant fused segment is apparent. In *C. cearensis* sp. nov. a clear division between segments I and II is present.

Araripus Carmo and Sampronha gen. nov.

<http://zoobank.org/58FB3D34-B53E-4735-8948-27371BB-740DA>

Type species. *Araripus crassitibialis* sp.nov.

Diagnosis. Large insects (length = 23.5 mm); head narrower than thorax; fore tibia swelled; frons slightly divergent at vertex and apparently not very broad; basal callus absent, but callosity visible at the dorsal half of frons; R_4 strongly sinuous at apex, parallel to R_{2+3} extremity; angle between R_4 and R_5 less than 90° ; vein r-m inclined anteriorly; abdomen very long, nearly twice the thorax length.

Etymology. From “Araripe”, the name of the sedimentary basin to which the Crato Formation belongs.

Comments. The new genus differs from other Cretaceous Tabanidae by the swelled tibia, the R_4 strongly curved to the wing base, and the unusually long abdomen.

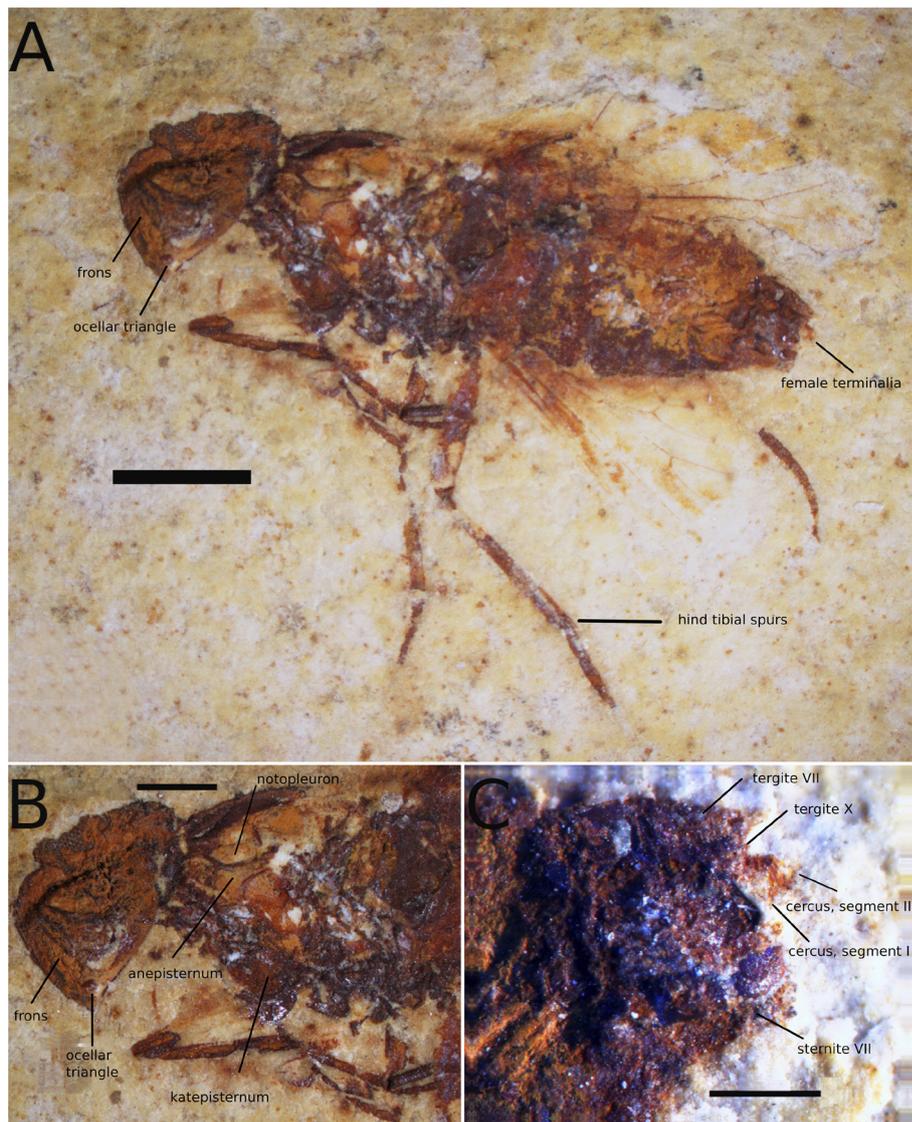


Figure 3. *Cratotabanus cearenis* sp.n. Holotype female. **A** Lateral habitus. **B** Head and thorax. **C** Terminalia. Scale bars: 2 mm (A); 1 mm (B); 0.5 mm (C)

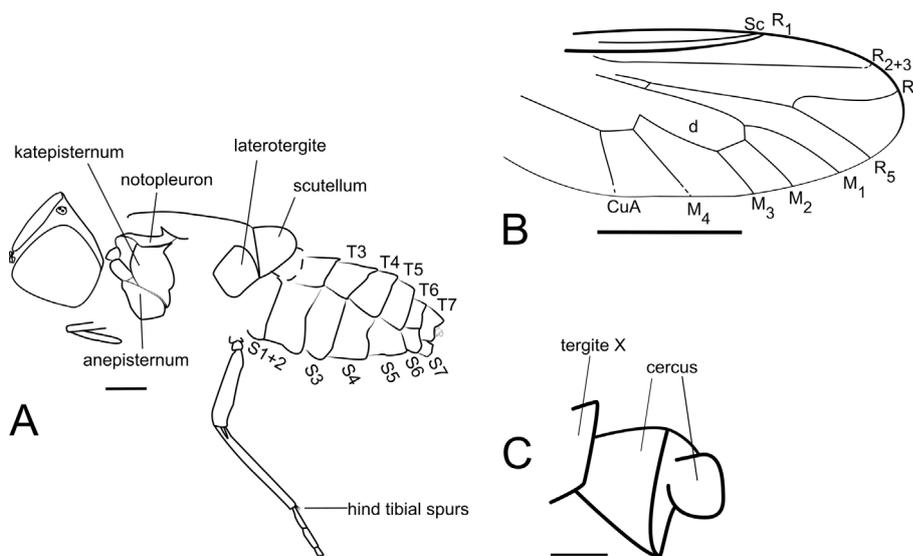


Figure 4. *Cratotabanus cearenis* sp.nov. Holotype female. **A** Illustration of lateral habitus. **B** Wing venation. **C** Female terminalia. Scale bars: 2 mm (A, B); 0.1 mm (C).

Araripus crassitibialis sp. nov. Carmo and Sampronha

<http://zoobank.org/BE39D533-FE52-42B7-870A-5D3B-0764D4E2>

Figs 5, 6

Examined material. *Holotype female*: GP1e/8751 NE Brazil, Crato Formation, Aptian, Lower Cretaceous.

Preservation. Preserved in dorsal view. Frons and occiput visible. Scutellum, notopleuron, one halter, foreleg preserved. Wings partially preserved. Tergites I–VII, tergite X and cercus visible.

Diagnosis. The same as the genus.

Description. *Holotype female.* Length 23.5 mm, wing 15.5 mm. – **Head:** hemispherical, narrower than thorax; scape oval, nearly as wide as long; frons appears divergent above; frontal callus absent; apparent callosity visible near the vertex; subcallus not inflated or pronounced; notopleuron robust and well developed. – **Thorax:** scutum and scutellum visible, the former much longer than wide. – **Legs:** fore tibia inflated. – **Wings:** vein Sc very long, inserting in C very close to R_1 , with few visible setae; pterostigma small, barely visible; R_{2+3} very sinuous, inserting in C parallel to R_4 and forming a 90° angle; vein R_4 strongly angled and without an appendix; cell r_5 open; vein M_1 sub-parallel to M_2 ; M_3 diverging from M_2 ; vein r-m inclined towards wing base; m-cu inserted very close to the origin of R_4 ; wing *ca.* 3 times longer than wide. – **Abdomen:** nearly twice the length of the thorax. – **Terminalia:** Tergite X appears to be undivided; cercus two-segmented.

Etymology. From latin, *crassus* (tick) + *tibia* (leg), in reference to the enlarged tibia.

Comments. The inflated tibia is present in several species from different tabanid genera, especially in the Chrysopsinae and Tabaninae subfamilies (Coscarón and Papavero 2009a), but had not been previously recorded for Cretaceous species. As in *Cratotabanus*, the female terminalia in *Araripus* **gen. nov.** has a two-segmented cercus. This is absent from every extant horse flies; in fact, the presence of a one-segmented cercus is synapomorphy of a clade formed by Athericidae + Tabanidae (Yeates 2002).

3.2. Character list

1. **Ocellar triangle:** (0) absent; (1) present.
2. **Second palpomere lateral sulcus:** (0) absent; (1) present.
3. **Proboscis length:** (0) longer than head height; (1) shorter than head height.
4. **Labella:** (0) fleshy; (1) slender.
5. **Eyes dorsal margin:** (0) straight; (1) curved.

6. **Ocelli:** (0) vestigial; (1) developed.
7. **Basal callus:** (0) absent; (1) present.
8. **Postpedicel:** (0) undifferentiated from other flagellomeres; (1) forming a basal plate derived from the fusion of proximal flagellomeres; (2) rounded, with terminal flagellomeres modified in an arista-like stylus.
9. **Scape:** (0) clearly longer than wide; (1) approximately as long as wide.
10. **Clypeus:** (0) rounded anteriorly; (1) flat anteriorly; (2) conical.
11. **Metathoracic scale:** (0) absent; (1) present.
12. **Hind tibial spurs:** (0) absent; (1) present.
13. **Costal vein:** (0) with normal width at base; (1) ticked at base.
14. **Vein Sc:** (0) bare; (1) setulose.
15. **Insertion of Vein R_{2+3} :** (0) close to R_1 ; (1) distant from R_1 .
16. **Shape of R_{2+3} :** (0) straight most of its length, curved only at the insertion in C; (1) with one or more sinuities on its length; (2) with a single strong concavity at the distal half.
17. **Cell r_4 :** (0) not encompassing the wing apex; (1) encompassing the wing apex.
18. **Origin of R_4 :** (0) anterior to the tip of M_3 ; (1) at the same level as the tip of M_3 ; (2) posterior to the tip of M_3 .
19. **Angle between R_4 and R_5 :** (0) lesser than 90 degrees; (1) 90 degrees.
20. **Lower calypter:** (0) reduced; (1) pronounced.
21. **Basicosta:** (0) bare; (1) setulose.
22. **Suprametacoxal connection:** (0) absent; (1) present.
23. **Type of suprametacoxal connection:** (0) reduced; (1) pronounced.
24. **Metacoxal pit:** (0) absent; (1) present.
25. **Cerci, female terminalia:** (0) one-segmented; (1) two-segmented.
26. **Tergite IX, female terminalia:** (0) divided into two separated triangular plates; (1) one single transverse bar.
27. **Tergite X, female terminalia:** (0) divided; (1) undivided.
28. **Mushroom shaped expansion in the spermatheca:** (0) absent; (1) present.
29. **Gonostyli posterior extremity:** (0) bipartite; (1) slender; (2) truncated; (3) rounded.
30. **Tergites IX+X, male terminalia:** (0) fused; (1) separated.
31. **Sagittal division of fused tergites IX+X, male terminalia:** (0) absent; (1) present.
32. **Epandrium articulation:** (0) free; (1) articulated on gonocoxites (Sinclair et al. 1994: character 12).
33. **Gonocoxal apodeme length:** (1) short at the most reaching anterior margin of hypandrium; (2) extending well beyond anterior margin of hypandrium (Sinclair et al. 1994; Kerr 2010).
34. **Endophalic tines:** (0) absent; (1) present.
35. **Endoedeagal process:** (0) absent or reduced; (1) present.

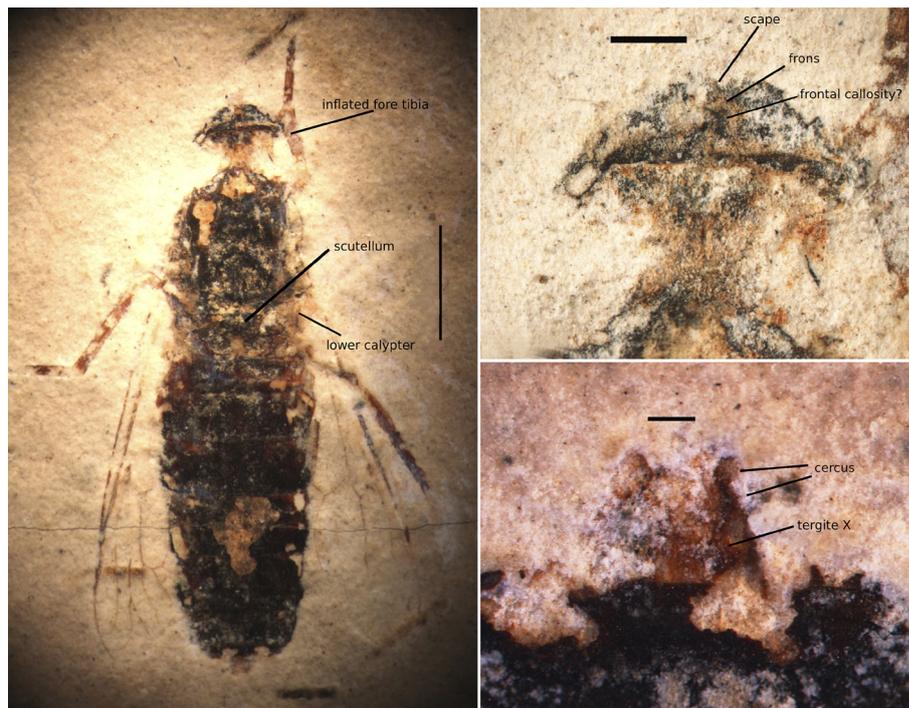


Figure 5. *Araripus crassitibialis* gen. nov. et sp. nov. Holotype female. **A** Habitus, preserved in dorsal view. **B** Head. **C** Terminalia. Scale bars: 5 mm (A); 1 mm (B); 0.2 mm (C).

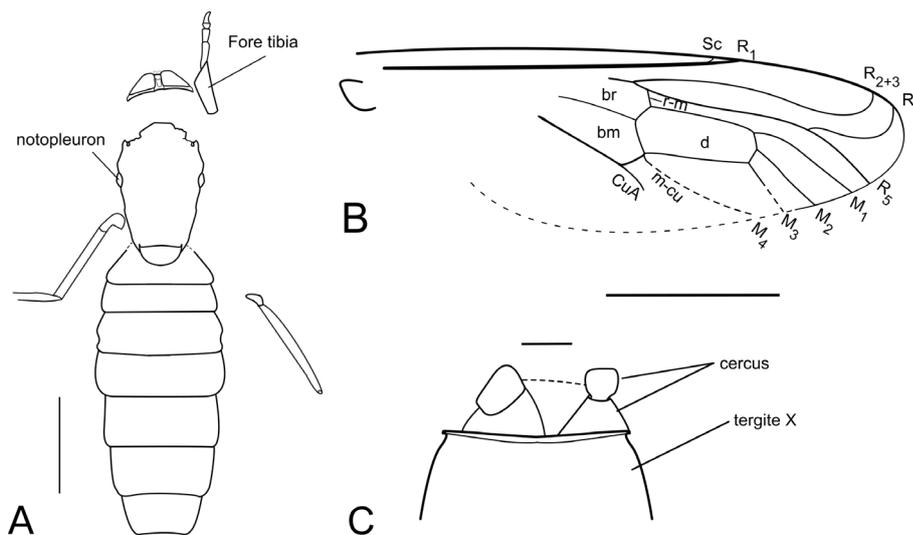


Figure 6. *Araripus crassitibialis* gen. nov. et sp. nov. Holotype female. **A** Illustration of habitus. **B** Wing venation. **C** Terminalia. Scale bars: 5 mm (A, B); 0.2 mm (C).

3.3. Phylogenetic analyses

The parsimony analysis using equal weights resulted in 2.630 most parsimonious trees with 56 steps, summarized in a strict consensus (Fig. 7A). Athericidae and Tabanidae were both recovered as monophyletic. The subfamilies Tabaninae and Chrysopsinae form a monophyletic group, which is congruent with molecular hypotheses (Morita et al. 2016). *Tabanipriscus transitivus* is sister group to all the remaining Tabanidae, while *Araripus* is sister group to all remaining horse flies, including *Cratotabanus*, which is located in a polytomy with a paraphyletic Pangoniinae. Parsimony analysis with implied weighting resulted in the same topology obtained with equal weights for all k values analyzed (5, 15, and 150).

In the Bayesian analysis (BI), chains reached convergence, with the standard deviation of the sampled splits observed to be 0.0038 and the trace plots reaching stationary distribution. Fig. 7B shows the topology and posterior probabilities (pp). The BI yielded results very similar to parsimony, however, *Cratotabanus* is in a polytomy at the base of a monophyletic crown group Tabanidae.

The resulting topologies of both optimality criteria employed are similar. For discussion of the evolution of traits, we plotted morphological characters in one of the most parsimonious trees obtained under equal weights. This tree was chosen as it summarizes results obtained under both Parsimony and Bayesian analyses. Clades recovered by different optimality criteria were indicated in this topology (Fig. 8).

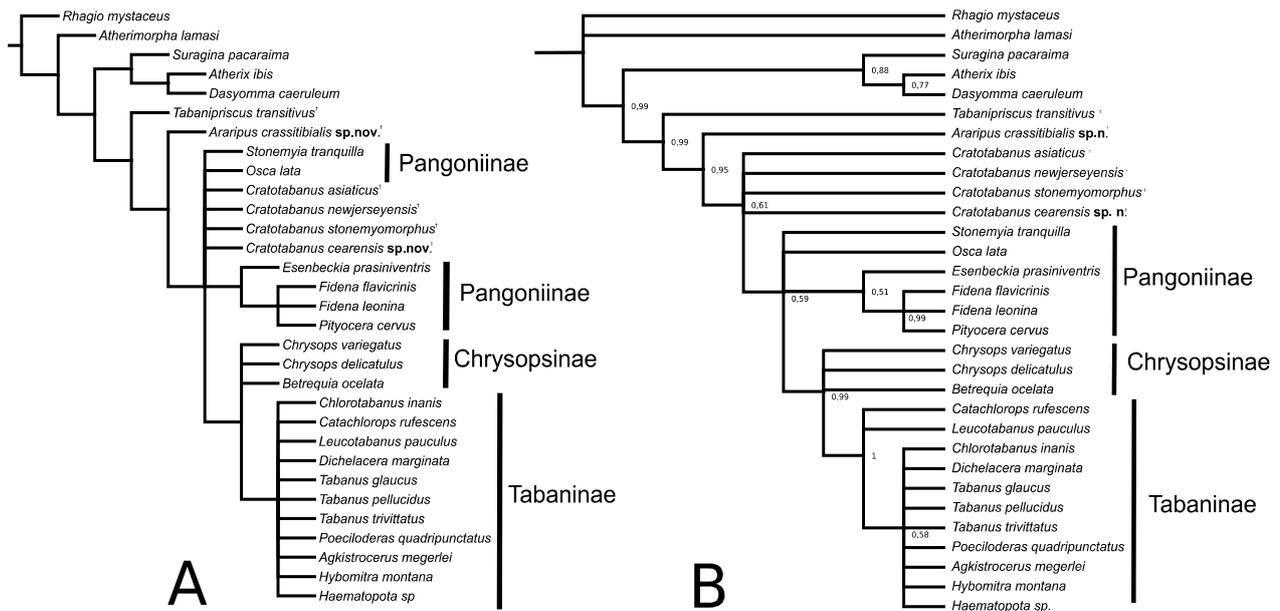


Figure 7. Phylogenetic relationships of fossil horse flies obtained under different optimality criteria. **A** Strict consensus from parsimony analysis with equal and implied weighting ($k = 5, 15$ and 150). **B** Bayesian Inferences, numbers over clades are posterior probabilities.

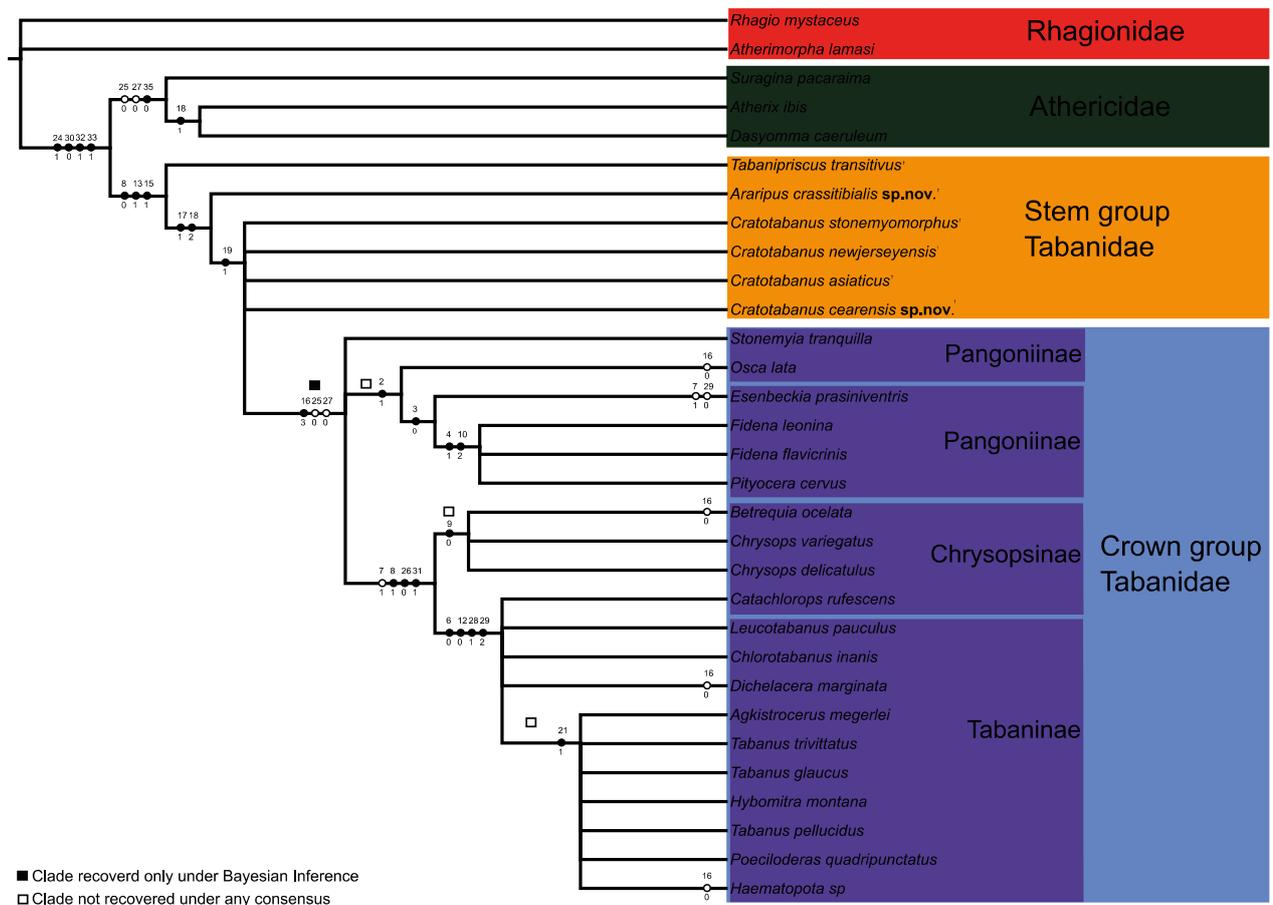


Figure 8. One of the most parsimonious trees obtained in the parsimony with equal weights, chosen to show the optimization of morphological characters. Clades obtained under different optimality criteria are indicated within the figure. Black circles represent unequivocal synapomorphies, while white circles represent homoplastic synapomorphies.

4. Discussion

4.1. Tabanidae

Both the monophyly of Tabanidae and its sister group relationship with Athericidae are well established in the literature (e.g. Yeates 2002; Wiegmann et al. 2011). Under both of the optimality criteria here employed, the sister group relationship between the two families is well supported. In the most parsimonious tree chosen as representative of our hypothesis, the clade Athericidae + Tabanidae is supported by four unequivocal synapomorphies: the fusion of tergites IX and X in the male [ch. 30(0)], a well-known characteristic of both families (Stuckenberg 1973; Woodley 1989); the presence of a suprametacoxal connection [ch. 24(1)]; the epandrium articulated freely with the gonocoxites [ch. 32(1)]; and gonocoxal apodemes extended beyond the hypandrium [ch. 33(1)].

In our analysis, Tabanidae is monophyletic, and the subfamilies Tabaninae and Chrysopsinae form a clade within Tabanidae. Chrysopsinae, however, appears as paraphyletic (Fig. 7). The monophyly of Chrysopsinae + Tabaninae is sustained by the presence of a conspicuous basal callus [ch. 7(1)]; the postpedicel fused with proximal flagellomeres in a basal plate [ch. 8(1)]; tergite IX of female divided in two triangular plates [ch. 26(0)]; and the sagittal division of fused tergites IX+X in the male terminalia [ch. 31(1)].

The Pangoniinae are recovered in a polytomy at the base of the Tabanidae crown-group (Fig. 8). In Morita et al. (2016), the Pangoniinae are monophyletic and sister group to the remaining subfamilies, with the Chrysopsinae paraphyletic relative to Tabaninae. The polytomy in the base of Tabanidae obtained in our results is possibly due to the reduced sample of each subfamily and the missing data introduced by fossil taxa. That said, it is not the objective of this paper to evaluate the internal relationship of Tabanidae, but, instead, to construct a framework to understand the evolution and phylogenetic positioning of fossil taxa using morphological characters. The fossil groups included in this analysis and their phylogenetic relationships are discussed in the sections below.

4.2. *Tabanipriscus* Grimaldi

The genus *Tabanipriscus* was described by Grimaldi (2016) based on a specimen from the Burmese amber. Despite not belonging to the Brazilian Cretaceous, we decided to include this genus in our analysis because it has similar traits to some Rhagionidae, Athericidae, and Tabanidae. According to Grimaldi (2016), *Tabanipriscus* could be a Tabanidae stem-group or sister group to Athericidae + Tabanidae. Our phylogenetic analysis supports the first hypothesis. In both optimality criteria employed, *Tabanipriscus* is sister to the clade *Araripus* + *Cratotabanus* + crown-group Tabanidae (Fig. 7). In the cladogram

of Fig. 8, *Tabanipriscus* shares with the other Tabanidae the proximal flagellomeres not fused into a postpedicel [ch. 8(0)], which is considered the plesiomorphic condition within Brachycera (Stuckenberg 1999). In Tabanidae, this condition is kept in most of the Pangoniinae genera, with different levels of fusion occurring in several groups, notably in the Tabaninae and Chrysopsinae. Other characters that support *Tabanipriscus* + Tabanidae include a vein C thickened at its basal portion [ch. 13(1)]; and R_{2+3} inserted in the costal vein far from R_1 [ch. 15(1)]. Both characters are recognized as diagnostic for Tabanidae (e.g. Stuckenberg 1973; Yeates 2002; Chainey 2017), with no known exceptions among living taxa. *Tabanipriscus* also lacks a metacoxal pit [ch. 22(1)] (Grimaldi 2016), considered diagnostic for Athericidae but absent from the athericid *Dasyomma* Macquart and all of the Tabanidae.

4.3. *Araripus* gen. nov.

The new genus described here, *Araripus* gen. nov., is sister to *Cratotabanus* + crown Tabanidae in both optimality criteria employed (Fig. 7). The monophyly of the clade *Araripus* gen. nov. + Tabanidae is sustained by the cell r_4 encompassing the wing apex [ch. 17(1)]; and the split of R_4 and R_5 posterior to the base of M_3 [ch. 18(2)] (Fig. 6B). Both characters are present in all of the extant Tabanidae, while the monophyly of the clade *Cratotabanus* + crown Tabanidae is sustained by the angle of 90 degrees at R_4 [ch. 19(1)], a character with known variation within the family. A well-defined ocellar triangle may not be distinguished in *Araripus crassitibialis* gen. nov. et sp. nov., and there is a dark structure near the vertex that could be a frontal callosity, but its position at the frons does not match the correspondent structures in extant horse flies. *Araripus* gen. nov. also has an inflated tibia, a characteristic that appears several times in both Tabaninae and Chrysopsinae subfamilies (Coscarón and Papavero 2009a; Chainey et al. 2017) but unknown in the Pangoniinae or in any other fossil previously described. Given its multiple appearances in species of different genera within Tabanidae, we did not include this character in our matrix.

4.4. *Cratotabanus* Martins-Neto and Santos

The genus *Cratotabanus* has currently four described species, two of them – *C. stonemyomorphus* and *C. cearensis* sp. nov. – from Brazilian Crato formation. The general form of the body and wing shape leaves little room for doubt about the identity of *Cratotabanus* as a Tabanidae (Bechly 2007; Grimaldi 2016). Nevertheless, some of the described species have characters considered plesiomorphies of Tabanomorpha, which raises interesting questions about the phylogenetic position of *Cratotabanus*. In our parsimony analysis (Fig. 7A), the genus is recovered within a polytomy at the base of Tabanidae

with species of the Pangoniinae subfamily, while the Bayesian Inference supports the species currently assigned to the genus in a polytomy basal to a monophyletic crown-group Tabanidae (Figs 7B and 8). The lack of preservation of diagnostic characters poses a challenge for understanding the evolution of *Cratotabanus*, since most characters that could be apomorphic for the genus are preserved in no more than two or three species. Still, our results confirm the relationship of these species with Tabanidae.

Three species of *Cratotabanus* (*C. asiaticus*, *C. stoneymorphus*, and *C. cearensis* **sp. nov.**) lack a basal callus [ch. 7(0)], a character present in only a few Pangoniinae but conspicuous in the Tabaninae and Chrysopsinae (e.g. Coscarón and Papavero 2009a). *Cratotabanus newjerseyensis*, the only species with preserved antennae, has seven flagellomeres, with a postpedicel undifferentiated from the remaining flagellomeres (Grimaldi et al. 2011). This character state is considered plesiomorphic for Tabanomorpha and present in most species of Pangoniinae with different levels of fusion in different groups (e.g. *Veprius* Rondani and *Zophina* Philip). The proximal segments of the flagellum form a basal plate of four to five fused flagellomeres in the Chrysopsinae and Tabaninae [ch. 8(1)]. The basicosta of *C. asiaticus* is bare (Grimaldi 2016: fig. 15A), a character state plesiomorphic in Tabanomorpha and in Tabanidae, while a basicosta with setae as dense as those in the costal vein is an apomorphy of the Tabanini tribe [ch. 21(1)].

Cratotabanus retains three important plesiomorphic characters of Tabanomorpha – the two-segmented cercus [ch. 25(1)]; an undivided tergite X [ch. 27(1)]; and the absence of a metathoracic postspiracular scale [ch. 11(0)]. The undivided tergite X in the female is seen in at least two species (*C. asiaticus* and *C. cearensis* **sp. nov.**) [ch. 27(1)]. In most Tabanidae, the tergite X in the female is divided, with the exception of the uncommon Pangoniinae genera *Archeomyotes* Philip and Coscarón, *Austromyans* Philip and Coscarón, *Fairchildimyia* Philip and Coscarón (Philip and Coscarón 1971) and some species of *Philoliche* Wiedemann (Stuckenberg 1973). Although not included in our analysis, these species are morphologically different among themselves and from *Cratotabanus*, indicating that a unique origin of the fusion of the tergite X in these groups is unlikely.

As *Tabanipriscus* and *Araripus* **gen. nov.**, the cerci of *Cratotabanus* are two-segmented. A one-segmented cercus is synapomorphy of Athericidae + Tabanidae while the plesiomorphic two-segmented condition is present in most families of Tabanomorpha. As with the undivided tergite X, our results suggest that a cercus with two segments is plesiomorphic in Tabanidae and that the cercus with one segment appears independently in Athericidae and the crown Tabanidae.

The postspiracular region is only visible in *C. asiaticus* (Grimaldi 2016). An absent or very reduced postspiracular scale (Grimaldi 2016) could be an apomorphy for the extinct genus, but further evidence is necessary to confirm this hypothesis.

5. Conclusions

The results presented here are relevant to the understanding of Tabanidae evolution and morphology. The new species described increase our knowledge about Cretaceous species, revealing new features previously not known from fossil species, like an inflated tibia. Also, several traits are seen in amber specimens of *Cratotabanus*, such as a two-segmented cercus and an undivided tergite X, which are also seen in Crato specimens, further supporting the idea of *Cratotabanus* as a genus.

The analysis undertaken here is also the first to evaluate the phylogenetic position of fossil horse flies, presenting evidence for Crato specimens as stem-Tabanidae and shedding light on the evolution of female terminalia. The position of the studied fossils as stem groups and the plesiomorphic traits displayed, unparalleled in extant taxa, also supports the idea of a late origin for modern horse fly subfamilies, a hypothesis already ventured elsewhere and supported by molecular divergence time estimations (Morita et al. 2016). The description of the first male horse fly of the Cretaceous also reveals an holoptic eye, a form of sexual dimorphism well known for the Tabanomorpha but also not registered for this period.

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Supplementary material 1

Examined extant specimens

Authors: Carmo DDD, Sampronha S, Santos CMD, Ribeiro GC (2022)

Data type: .ods

Explanation note: This file includes extant specimens examined for phylogenetic analysis.

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Supplementary material 2

Morphological matrix 1

Authors: Carmo DDD, Sampronha S, Santos CMD, Ribeiro GC (2022)

Data type: .pdf

Explanation note: Morphological matrix in Nexus format with Mrbayes datablock.

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Supplementary material 3

Morphological matrix 2

Authors: Carmo DDD, Sampronha S, Santos CMD, Ribeiro GC (2022)

Data type: .pdf

Explanation note: Morphological matrix in nona format.

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