

Cladistic analysis of Egyptian horse flies (Diptera: Tabanidae) based on morphological data

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ABSTRACT

The family Tabanidae is one of the important families of superfamily Tabanoidea, having medical and veterinary importance. In Egypt, there is no strict cladistic approach on tabanid flies phylogeny yet. The 20 available Egyptian tabanids under 2 subfamilies are analyzed cladistically. Cladistic analysis is based on 91 morphological characters depending on Single linkage, UPGMA, Complete linkage clustering methods (Cophenetic correlation value). This produces a well-resolved and firmly supported phylogenetic hypothesis on the generic relationships. Based on our phylogenetic results, the revised classification of examined taxa is consistent with the conventional classification.

Key words: Morphology, Phylogeny, tabanid flies, Cladistic analysis, Egypt.

INTRODUCTION

Tabanidae is a cosmopolitan family and one of the large Brachyceran flies, comprising almost 4400 world-wide species within 144 genera (Evenhuis *et al.*, 2008). Tabanid flies are blood feeding and important vectors of diseases to human and livestock such as surra, anthrax and *Loa loa* (Mullens, 2009).

Despite of the economic and medical importance of Tabanidae, taxonomy within the family has been historically intractable (Oldroyd 1957; Chainey 1993), and there is a lack of knowledge about phylogenetic relationships among different taxa. As (Mackerras *et al.*, 2008) stated, they are among the least understood fly families in terms of modern phylogeny based classifications or recent global monographic coverage.

The dependence on the color pattern must be used with caution in partially denuded specimens (Pechuman, 1972)). Thus, identification is not easy in tabanids due to change of external colors depending on the methods of collection and preservation and the time duration of preserved specimens. Chvála *et al.* (1972) mentioned that the genitalia are important character for the classification of higher categories of the family, but it can not be used in the separation of genera and species.

Most current authors accept Mackerras' tabanid classification which based on morphological characters (1954, 1955a, 1955b) and adopt the following subfamilies and tribes: Chrysopsinae (Bouvieromyiini, Chrysopsini, Rhinomyzini), Tabaninae (Diachlorini, Haematopotini, Tabanini), and Pangoniinae (Pangoniini, Philolichini, Scionini) (Chainey, 1993 and Mackerras *et al.*, 2008).

In Egypt, the family Tabanidae was early studied by Kröber (1925) describing 22 tabanid species within 3 genera and 3 subgenera. Efflatoun (1930) published a

monograph of Egyptian Tabanidae with 15 described species including one new species. Currently, tabanid flies were represented by 30 species and one variety within 5 genera according to the list of Steyskal and El-Bialy, 1967. In addition, Ahmed (1991) studied the blood sucking flies of order Diptera (except mosquitoes) including family Tabanidae. He described the same Efflatoun species and misidentified 2 species in his work.

There is a growing interest in mapping comparative morphological data onto phylogenies to provide morphological characters for identification purposes and to help in the placement of taxa in the correct taxonomic positions.

Accordingly, this work presented the first cladistic analysis and the first cladogram to Egyptian Tabanidae. Also, it aims to conduct a phylogenetic framework among the different taxa within the family.

MATERIAL AND METHODS

Taxon sampling

Twenty available species (Appendix 1) belonging to 5 genera and 2 subfamilies (Chrysopsinae and Tabaninae) of family Tabanidae from Egypt were examined. The taxa sampling aim to reflect the diversity encountered in the family. The third subfamily Pangoniinae (one species) not included in the analysis because it is not represented in the Egyptian collections and not collected from the field during the study.

The specimens examined for this analysis included the species which were collected from different localities such as El Khanka (Qalubiya), Ashmoon (Monophya), Alexandria, Kharga oasis and Bahariya oasis and those which belonging to the 5 main Egyptian Reference Collections:

(ASUC): the Collection of Ain Shams University, Faculty of Science, Entomology Department.

(CUC): the Collection of Cairo University, Faculty of Science, Entomology Department.

(ESEC): the Collection of Entomological Society of Egypt.

(AZUC): the Collection of Alfieri, Al Azhar University, Faculty of Agriculture.

(MAC): the Collection of the Ministry of Agriculture, Plant Protection Institute, Section of Identification.

Classification

The classification used in the paper and species identification generally follow Austen (1920), Kröber (1925), Efflatoun (1930), Oldroyd (1952, 1954), Mackerras (1954, 1955a, 1955b), Chvála *et al.* (1972) and Wilkerson *et al.* (1985). Morphological terminologies follow Verrall, 1909, Efflatoun (1930), Oldroyd (1952, 1954), Chvála *et al.* (1972) and Axtell (1976).

Characters selection

Total of 91 adult morphological characters with 182 character states of 20 taxa (OTUs, Operational Taxonomic Units) are used in data matrix (Appendix 3) to show the similarity matrix (Appendix 4). The Characters include both qualitative and quantitative type characters to increase the reality of the results.

Measurements of insect body parts were made with a calibrated ocular lens standardized at 100 units (ocular micrometer) using a stereomicroscope at magnification 100x to 400x

The characters and their states are listed below (Appendix 2). The non-compared characters are coded by (?).

Data analysis

Cladistic analysis is elaborated by program [PROBIOSYS, version 1.0, (2003)] depending on Single linkage, UPGMA (unweighted pair-group method analysis), Complete linkage clustering methods in numerical taxonomy, Cophenetic correlation value .

RESULTS

The resulted cladogram (Appendix 5) shows two main clusters at a similarity level of 51.8 %. The first contains only *Chrysops* sp. which was linked to the next major cluster that contains the other 19 species which included in the analysis. *Chrysops* sp. was clearly separated from the other tabanid species according to the following characters: hind tibia with apical spin, presence of ocelli, eyes in ♂ semi-contiguous, scape & pedicel elongated and slender, pedicel as long as scape, length of basal antennal segments more than (1/2 length of antennae, length of flagellum & 2 times width of scape), length of antennae more than 2 times length of flagellum, face with genal, rostral & facial calli and abdominal pattern differ in both sexes.

From the second major cluster, *Haematopota minuscula* separated from the other 18 tabanids at a similarity percentage 58.7 % based on the characters: wing with rosettes shape maculae, scape swollen, basal flagellomere without dorsal hump, width of basal flagellomere less than 1/2 width of scape, style as long as 1/4 length of antennae and in female, frons as long as broad, with one lower callus & with 3 rounded velvety black spots.

The 18 species was divided into 2 clades at similarity level 66.6%. The first clade includes 2 species, *Tabanus biguttatus* and *Dasyrhamphis nigrinus* based on maculated wing and separated from each other at similarity level 83.8 % according to the presence or absence of hairs on eyes and basicosta, number of distinct calli, shape of middle callus, fusion of lower and middle calli, and abdomen with or without patterns. The second clade contains 16 species. *Atylotus aegyptiacus* evolved early out of the 16 species at similarity level 73.3 % according to characters: abdomen without patterns and basal antennal segments whitish color.

The rest 15 species of the second clade at similarity level 75.1 % split into 2 large monophyletic groups. The first large monophyletic group includes 7 species: *Tabanus albifacies*, *T. separatus*, *T. lunatus*, *T. cordiger*, *T. mordax*, *T. arenivagus* and *T. sufis*. This group divided into 2 subgroups at similarity level 76.4 % . The first subgroup includes two species, *T. arenivagus* and *T. sufis* that shared based on the characters: subcallus more or less shining on upper part only and width of basal flagellomere nearly equal 1/4 length of flagellum. At similarity percentage 88.2 %, *T. sufis* is distinguished from *T. arenivagus* based on the presence or absence of R₄ appendix, frontal index, number of stripes on thorax, design of abdominal patterns, presence of upper callus, color of style and proportion between width of thorax to width of head.

The second subgroup includes 5 species and separated into 2 main branches at similarity level 80.2 %. The first branch includes 2 species, *T. cordiger* and *T. mordax* sharing on the following characters; presence of parafacial band and style & basal antennal segments black color. At similarity percentage 86.2 %, *T. mordax* differentiated from *T. cordiger* according to the shape of subcallus & middle callus, color of basal flagellomere, length of body in ♀ and proportion between width of head to (length of head & width of thorax). The second branch included 3 species, *T. albifacies*, *T. separatus* and *T. lunatus*. *T. lunatus* evolved out from the second branch

at similarity index 81.9 % according to the characters: hairy eyes, basal antennal segments & basal flagellomere orange color, R₄ vein without appendix, height of head more than 1/2 width of head, frons with three distinct calli, thorax with 3 stripes, width of thorax more than 3/4 width of head and abdomen olive grey with 3 greyish longitudinal stripes. *T. albifacies* and *T. separatus* are closely related to each other at similarity level 93 % but differ according to the proportion between width of head to (height of head & width of thorax) and color of basal antennal segments.

The second large monophyletic group includes 8 species, *Tabanus autumnalis*, *T. gratus*, *T. taeniola*, *T. rupinae*, *Atylotus agrestis*, *A. farinosus*, *A. pulchellus* and *A. agricola*. At similarity percentage 78.2 %, this group divided into 2 subgroups. The first subgroup includes 5 species that differentiated from the other subgroup according to the characters: R₄ with appendix and all antennal segments with the same color. *Tabanus rupinae* is split out at similarity level 80.9 % according to these combination of characters: width of head (more than 2 times length of head & more than 2 times height of head), parafacial band present, hairs of basicosta dark colored, middle & lower calli jointed, middle callus broader than 3/4 width of frons & crescent shape, lower callus wider than 1/2 width of frons, wing veins dark brownish to blackish and thorax with 3 stripes. *Atylotus agricola* is evolved out from *Atylotus* spp. at similarity level 91.2 % according to the characters: eyes hairy, abdomen orange yellow color with blackish median stripe, body length in ♀ shorter than 14.5 mm and length of head less than height of head. The other 3 species can be divided into 2 branches at similarity level 94.6 %, the first branch includes *A. pulchellus* based on the characters: wing veins yellowish on basal half and brownish on apical half and abdomen greyish color with 4 dark longitudinal stripes. The second branch includes two species, *A. agrestis* and *A. farinosus* which are closely related to each other at similarity level 96.3 % but differ in the characters: proportion between width of head to width of thorax, margins of frontal stripe parallel or not and thorax with or without stripes. The second subgroup includes 3 species, *T. autumnalis*, *T. gratus* and *T. taeniola*. *T. autumnalis* evolved out at similarity percentage 83.6 % according to the following characters: body length in ♂ more than 17 mm, length of head less than 3/4 height of head, width of head less than 1.25 width of thorax, pedicel dark reddish brown to blackish color, frons with 2 calli, middle and lower calli jointed and middle callus linear shape. At similarity level 88.5 %, *T. taeniola* is differentiated from *T. gratus* based on the characters: body length in ♀, color of style, margins of frontal stripe parallel or not, presence of upper callus, shape and width of middle callus and design of abdominal patterns.

DISCUSSION

The results of the current cladistic analysis increased our understanding of the phylogenetic relationships between the genera and species of family Tabanidae in Egypt. In general, these results are most consistent with the conventional classification of the family; *Chrysops* sp. (subfamily Chrysopsinae Lutz) is early split from the all other species -included in the analysis- which belonging to subfamily Tabaninae Loew. *Haematopota minuscula* (tribe Haematopotini Bequaert) was early evolved out from the rest species. After that, *Dasyrhamphis nigrinus* (tribe Diachlorini Lutz) was separated. The rest species belonging to the two genera *Tabanus* Linnaeus and *Atylotus* Osten-Sacken (tribe Tabanini Enderlein) were grouped together as shown in the cladogram. The only surprising result of the analysis is the deep position of *Tabanus biguttatus* as sister taxon to *Dasyrhamphis nigrinus* at similarity level 83.8

%. The only character that shared between the two species and which responsible for the deep position of *T. biguttatus* is the maculated wing. In fact, the only valid character that differentiated between the two tribes (Tabanini and Diachlorini) is the presence or absence of hairs on basicosta which is not enough. Unfortunately, we can not improve this character due to our fauna comprises only 3 genera of the two tribes.

Thus, in conclusion, we considered the position of this taxon as tentative and this hypothesis should be verified by addition of more terminal taxa in future works.

ACKNOWLEDGMENTS

We thank the staff members of the institutions listed in the materials section for their kind help. Our appreciation is given to Prof. Dr. Stjepan Krčmar, head of Department of Zoology, Division of Biology, Josip Juraj Strossmayer University of Osijek, (Croatia) for providing specimens and important literatures for our work.

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Appendix 1

List of the genera and species included in the analysis.

Chrysops Meigen, 1803.

Chrysops sp.

Tabanus Linnaeus, 1758.

T. albifacies Loew, 1856.

T. arenivagus Austen, 1920.

T. autumnalis Linnaeus, 1761.

T. biguttatus Wiedemann, 1830.

T. cordiger Meigen, 1820.

T. gratus Loew, 1858.

T. lunatus Fabricius, 1794.

T. mordax Austen, 1911.

T. rupinae Austen, 1920.

T. separatus Efflatoun, 1930.

T. sufis Jaennicke, 1867.

T. taeniola Palisot de Beauvois, 1807.

Atylotus Osten-Sacken, 1876.

A. aegyptiacus [Kröber, 1925].

A. agrestis [Wiedemann, 1828].

A. agricola [Wiedemann, 1828].

A. farinosus [Szilády, 1915].

A. pulchellus [Loew, 1858].

Haematopota Meigen, 1803.

H. minuscula Austen, 1920.

Dasyrhamphis Enderlein, 1922.

D. nigrinus [Fabricius, 1794].

Appendix 2

Characters and characters states

1. Body length in (♂): (-) = shorter than 17 mm, (+) = longer than 17 mm.
2. Body length in (♀): (-) = shorter than 14.5 mm, (+) = longer than 14.5 mm.
3. Ocelli: (-) = absent, (+) = present.
4. Eyes: (-) = bare, (+) = hairy.
5. Eyes in (♂): (-) = contiguous, (+) = semi-contiguous.
6. Width of head: (-) = less than 2 times length of head, (+) = more than 2 times length of head.
7. Length of head: (-) = less than 3/4 height of head, (+) = more than or equal to 3/4 height of head.
8. Height of head: (-) = less than half width of head, (+) = more than or equal to half width of head.
9. Proportion between width of head to width of thorax in (♂): (-) = less than 1.25, (+) = more than 1.25.
10. Proportion between width of head to width of thorax in (♀): (-) = less than 1.25, (+) = more than or equal to 1.25.
11. Length of antennae: (-) = less than or nearly equal to 3/4 length of head, (+) = more than 3/4 length of head.
12. Proportion between length of antennae and length of flagellum: (-) = less than 2, (+) = more than 2.

13. Length of scape & pedicel: (-) = less than half length of antennae, (+) = more than half length of antennae.
14. Length of antennal flagellum: (-) = less than length of basal antennal segments, (+) = more than length of basal antennal segments.
15. Antennal scape & pedicel: (-) = with different colors, (+) = with the same color.
16. Antennal scape & pedicel whitish color: (-) = absent, (+) = present.
17. Color of antennae: (-) = antennal segments with different colors, (+) = all antennal segments with the same color.
18. Antennal scape blackish color: (-) = absent, (+) = present.
19. Antennal scape brownish grey color: (-) = absent, (+) = present.
20. Antennal scape reddish yellow to reddish or yellowish brown color: (-) = absent, (+) = present.
21. Width of scape: (-) = less than half length of basal antennal segments, (+) = more than half length of basal antennal segments.
22. Antennal scape cup shaped: (-) = absent, (+) = present.
23. Antennal scape swollen: (-) = absent, (+) = present.
24. Antennal scape elongated and slender: (-) = absent, (+) = present.
25. Length of pedicel: (-) = shorter than scape, (+) = as long as scape.
26. Antennal pedicel: (-) = elongated slender, (+) = cup shape.
27. Antennal pedicel reddish yellow to reddish or yellowish brown color: (-) = absent, (+) = present.
28. Antennal pedicel dark reddish brown to blackish color: (-) = absent, (+) = present.
29. Antennal pedicel black color: (-) = absent, (+) = present.
30. Antennal scape, pedicel & basal flagellomere orange color: (-) = absent, (+) = present.
31. Proportion between width of basal flagellomere & width of scape: (-) = less than or equal to $1/2$, (+) = more than $1/2$.
32. Width of basal flagellomere: (-) = less than or nearly equal to $1/4$ length of flagellum, (+) = more than $1/4$ length of flagellum.
33. Basal flagellomere: (-) = without dorsal hump, (+) = with dorsal hump.
34. Basal flagellomere dark brown color: (-) = absent, (+) = present.
35. Basal flagellomere reddish yellow to reddish or yellowish brown color: (-) = absent, (+) = present.
36. Basal flagellomere black color: (-) = absent, (+) = present.
37. Color of style with the same color of basal antennal segments: (-) = absent, (+) = present.
38. Length of style: (-) = as long as or less than $1/4$ length of antennae, (+) = more than $1/4$ length of antennae.
39. Color of style reddish yellow: (-) = absent, (+) = present.
40. Color of style dark brown: (-) = absent, (+) = present.
41. Color of style black: (-) = absent, (+) = present.
42. Face with genal, rostral & facial calli: (-) = absent, (+) = present.
43. Subcallus swollen: (-) = absent, (+) = present.
44. Subcallus entirely bare & shining: (-) = absent, (+) = present.
45. Subcallus entirely dull and tomented: (-) = absent, (+) = present.
46. Subcallus more or less shining on the upper part only: (-) = absent, (+) = present.
47. Parafacial band: (-) = absent, (+) = present.

48. Margins of frontal stripe in (♀): (-) = not parallel, (+) = parallel.
49. Frons in (♀): (-) = as long as broad, (+) = distinctly longer than broad.
50. Width of frons in (♀): (-) = less than 1/3 width of head, (+) = more than 1/3 width of head.
51. Frontal index in (♀): (-) = less than 1.7, (+) = more than 1.7.
52. Frons in (♀) with only one lower callus: (-) = absent, (+) = present.
53. Frons in (♀) with only two calli (lower & middle): (-) = absent, (+) = present.
54. Frons in (♀) with lower, middle & upper calli: (-) = absent, (+) = present.
55. Frons in (♀) with 3 rounded velvety black spots at middle: (-) = absent, (+) = present.
56. Upper callus in (♀): (-) = vestigial, (+) = distinct.
57. Middle & lower calli in (♀): (-) = separated, (+) = jointed to each other.
58. Upper & middle calli in (♀): (-) = separated, (+) = jointed to each other.
59. Width of lower callus in (♀): (-) = as wide as or less than half width of frons, (+) = wider than half width of frons.
60. Middle callus in (♀): (-) = narrower than 3/4 width of frons, (+) = broader than 3/4 width of frons.
61. Shape of middle callus in (♀): (-) = sub-linear or linear, (+) = not linear (oval, rounded, heart shape, elongated, quadrate shape or crescent shape).
62. Middle callus in (♀) oval shape: (-) = absent, (+) = present.
63. Middle callus in (♀) heart shape: (-) = absent, (+) = present.
64. Middle callus in (♀) crescent shape: (-) = absent, (+) = present.
65. Middle callus in (♀) semi-quadrate to quadrate shape: (-) = absent, (+) = present.
66. Middle callus in (♀) elongated: (-) = absent, (+) = present.
67. Thorax: (-) = not striped, (+) = striped.
68. Thorax with 3 stripes: (-) = absent, (+) = present.
69. Thorax with 4 stripes: (-) = absent, (+) = present.
70. Thorax with 5 stripes: (-) = absent, (+) = present.
71. Width of thorax: (-) = less than 3/4 width of head, (+) = equal to or more than 3/4 width of head.
72. Hind tibia: (-) = without apical spin, (+) = with apical spin.
73. Wing: (-) = not maculated, (+) = maculated.
74. Maculated wing: (-) = partially covered with maculae, (+) = completely covered with maculae.
75. Wing with rosettes-shape: (-) = present, (+) = absent.
76. Wing veins yellow color: (-) = absent, (+) = present.
77. Wing veins dark brownish to blackish color: (-) = absent, (+) = present.
78. Wing veins yellowish on basal half and brownish on apical half: (-) = absent, (+) = present.
79. R₄ vein: (-) = without appendix, (+) = with appendix
80. Basicosta: (-) = without hairs, (+) = with hairs.
81. Hairs of basicosta: (-) = pale colored, (+) = dark colored.
82. Abdomen: (-) = without patterns, (+) = with patterns.
83. Abdominal patterns: (-) = not similar in both sexes, (+) = similar in both sexes.
84. Abdomen orange yellow color with blackish median longitudinal stripe: (-) = absent, (+) = present.
85. Abdomen whitish or grayish color with 4 dark longitudinal stripes: (-) = absent, (+) = present.

86. Abdomen reddish brown color: (-) = with broad blackish median stripe & 2 lateral narrow blackish stripes, (+) = with triangles greyish median stripe & 2 oval greyish sublateral stripes.
87. Abdomen olive grey with 3 greyish longitudinal stripes: (-) = absent, (+) = present.
88. Abdomen reddish yellow color: (-) = with 3 dark longitudinal stripes, (+) = with 4 dark longitudinal stripes.
89. Abdomen blackish color: (-) = with 2 yellow triangular spots on terga 3 & 4, (+) = with 3 greyish longitudinal stripes.
90. Abdomen in ♀ greyish yellow with black spots on terga 1 & 2 and black seams on sides of anterior margin of terga 3 & 4: (-) = absent, (+) = present.
91. Abdomen in ♂ yellow with black spots: (-) = absent, (+) = present.

ARABIC SUMMARY

تحليل العلاقات التطورية لذباب الخيل (ثنائية الأجنحة – تابانيدى) فى مصر باستخدام الصفات المورفولوجية

جوهرة مجدى محمد أبو الحسن - هيثم بدرأوى موسى بدرأوى- سلوى كمال محمد - حسن حمدنا الله فضل
قسم علم الحشرات - كلية العلوم - جامعة عين شمس - العباسية - القاهرة- مصر.

تعتبر فصيلة تابانيدى واحدة من أهم الفصائل فى فوق فصيلة تابانويديا لكونها ذات أهمية طبية و بيطرية . و حتى الان لا يوجد أى دراسات لتحديد العلاقات التطورية بين ذباب التابانيدى فى مصر . فتنضم الدراسة تطبيق البرنامج الحاسوبى "PROBIOSYS" لتحديد العلاقات التطورية بين الأنواع المتاحة من الفصيلة و هم عشرون نوعا باستخدام 91 صفة مورفولوجية. و لقد توصلت الدراسة الى بناء العلاقات التطورية بين الأنواع و التى جاءت متوافقة الى حد كبير مع نتائج التصنيف التقليدى للفصيلة.