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Cytogenetic Analysis of the Sudanese Horse Flies (Diptera: Tabanidae) from Gedaref State, Eastern Sudan

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Abstract
The Sudanese Horse flies are a group of hematophagous insects that are responsible for transmitting diseases to animals. A cytogenetic analysis of field–collected Tabanids flies, was carried out in Al-Showak district, Gedaref state, Eastern Sudan, to have information on the karyotypic structures prevalent in the area. In this study the karyotype of five horse flies species, *Tabanus autumnalis* (Szilády 1914); *Tabanus leleani* (Austen 1920); *Atylotus pulchellus* (Loew 1858); *Hybomitra mendica* (Villeneuve 1912) and *Tabanus sp.* were described for the first time in Sudan. Adult flies are collected by the trapping method; all specimens are transferred in to a preservative solution to the laboratory, where they firstly, morphologic and morphometric identified. Flies are dissected, fixed, stained and slides are prepared by squashing method, examined under the light microscope and photographed. Cytogenetic analysis showed the presence of five different pattern of karyotype in the collected Tabanids samples; *Tabanus autumnalis* (Szilády 1914) 7n = 14; *Tabanus leleani* (Austen 1920) 5n = 10; *Atylotus pulchellus* (Loew 1858) 6n = 12; *Hybomitra mendica* (Villeneuve 1912) 9n = 18 and *Tabanus sp.* 8n = 16. This study showed the presence of two genetically differentiated populations of Tabanus species as sympatric in Gedaref region. This karyotypic information will help in understanding the inheritance of the phenotypic variation within the horse flies and will be useful as taxonomic tool.

1. Introduction

The Tabanidae are true flies and members of the insect order Diptera [9, 25] with the families Athericidae, Pelecorhtnchidae and Oredeptidae, Tabanidae are classified in the superfamily Tabanoidea. Along with Rhagionoidea, this superfamily makes up the infra-order Tabanomorpha. The family Tabanidae includes approximately 4300 to 4500

species and subspecies worldwide. Of these, 335 species in 25 genera are found in the Nearctic Region [8], over 1,300 of the genus Tabanus [24]. The family Tabanidae is divided in to three subfamilies: Chrysopinae, Tabaninae and Pangoniinae [12, 19]. The former two of these subfamilies contain most of the economically important tabanids. Tabanids in the subfamily Chrysopinae are called deerflies, with nearly all being members of the genus Chrysops. The subfamily Tabaninae includes horse flies, represented by the genera *Tabanus* and *Hybomitra*.

Females of most Tabanidae species attack mammals, principally Equidae, Bovidae, Camelidae, and humans. To obtain the blood, the females bite animals including humans, while the males are harmless, it takes the female about six days to fully digest its blood meal, and after that, it needs to find another host [21]. Saliva containing anticoagulant is injected in to the wound to prevent clotting [16]. Like other blood-sucking flies, tabanids have an economic effect on milk production where they commonly live [9]. Moreover, tabanids are known worldwide as important mechanical vectors of virus, bacteria, protozoans and helminths, which cause diseases in some wild and domestic animals [11, 18, 10, 4, and 13].

Tabanid control is difficult to achieve. A given area typically has multiple species with different seasonal occurrences and biological characteristics. Typical host contact is only about 4 min. per fly during blood feeding, which may occur only once every 3-4 days.

Tabanids are attracted to a variety of visual cues including color and shape [1] whereas contrasting colors play a role in tabanid attraction to two-dimensional panels, these flies appear to be more attracted to dark 3-dimensional objects [2, 7]. Synthetic attractants in traps are used in many parts of the world to collect female tabanids [27]. Efficacy of tabanid traps is mainly increased by the addition of chemicals that mimic natural host odors [14]. Some natural attractants such as the aged urine of cows, African buffalo, horses, and rhinocero s are used mainly for tsetse species (Glossinidae) andTabanidae [28, 20, 17, and 23].

Mitotic or meiotic divisions in ovaries or testes of adult tabanids are rarely found. Females of many species require a blood meal to complete ovarian development and in most of them this intake is necessary to end subsequent rest periods during the oviposition cycle. However, unlike females, males don't need blood feeding, and testes develop in a very short period [9, 29, 10, and 33].

Applications of chromosome analysis are essential because of the advantages of karyotypic analysis as taxonomic characters; particularly for agricultural and forest pests and disease vectors. However, scarce studies concerning the chromosomal diversity of horse flies are found in the literature. Therefore, the objective of this paper was to investigate the karyotype pattern of the horse flies in Al-Showak district, Gedaref state, Eastern Sudan.

2. Materials & Methods

2.1. Study Area

Gedaref State

This state is located in the Eastern Sudan, and it extends over the large savannah region. The climate is a tropical continental with two seasons; rainy season lasting from June–October and dry season between November to May with an average min–max temperature of 34-40°C. The average annual rain fall is 815mm. Horse flies are collected from Al-Showak (14°25'N-35°52'E). Gadref state is characterized by rich vegetation, particularly during the rainy season. The main trees and bushes include; *Balanites aegyptiaca*, *Acacia seyal*, *Acacia mellifera*, *Acacia nilotica*, *Acacia senegal*, *Combretum spp.*, *Azadirachta indica* and *Ziziphus spina-christi*.

2.2. Field Collection of Tabanids

Showak area is selected for the collection of Tabanids flies. The Tabanidae are collected by the trapping method. In this study, tabanids are caught by using two types of blue/black clothes (Nettings), NZ1 traps and H-trap and two types of natural attractive odor, urine, and stool of camel were placed for the flies. Some 47 samples were collected in October 2014 from Al-Showak, transferred to the Laboratory where they are morphologic and morphometric identified, and are stored in the fridge until cytogenetic analysis on June 2015.

2.3. Preparation of Mitotic Chromosomes

The collected flies were transferred to the laboratory in Carnoy fixative (3:1 Ethanol 70%: Glacial acetic acid 45%) and stored at 4°C. Later, ovarian tissue of females and testis of males were dissected carefully in hypotonic sodium citrate solution (1% in dH_2O) in room temperature and transferred again in Carnoy fixative (3: 1 Ethanol 70%: Glacial acetic acid 45%). These were deposited in this solution and incubated at –20°C for 24 hours. After fixation, tissues were washed with dH_2O and relocated in 0,1 NHCl, 20°C. Tissues were washed again with dH_2O, and a suspension is prepared in one drop of 2% aceto-orcein on a microscope slide for a period of 10 minutes for staining purpose. Then, the cover glass was placed over it and sealed with colorless nail polish. The slides were observed by a research microscope using 100x magnifications and photographed.

3. Results

To obtain data on chromosome morphology, twelve plates of mitotic preparations were analyzed and evaluated for each sample. *Tabanus autumnalis* (Szilády 1914) (Sh-T13) has shown seven pairs of chromosomes from ovarian cells of females and testis of males (Figure 1).

Table 1. *Chromosome numbers of some Tabanidae species collected in this study.*

Sample Code	Number of Chromosomes	Tabanid Species
Sh-T7	7n (14)	*Tabanus sp.*
Sh-T8	8n (16)	*Tabanus sp.*
Sh-T11	6n (12)	*Tabanus sp.*
Sh-T13	7n (14)	*Tabanus sp.*
Sh-T14	8n (16)	*Tabanus sp.*
Sh-T16	9n (18)	*Hybomitra sp.*
Sh-T17	6n (12)	*Hybomitra sp.*
Sh-T19	7n (14)	*Tabanus sp.*
Sh-T23	7n (14)	*Tabanus sp.*
Sh-T39	6n (12)	*Hybomitra sp.*
Sh-T40	6n (12)	*Atylotus sp.*
Sh-T41	5n (10)	*Tabanus sp.*

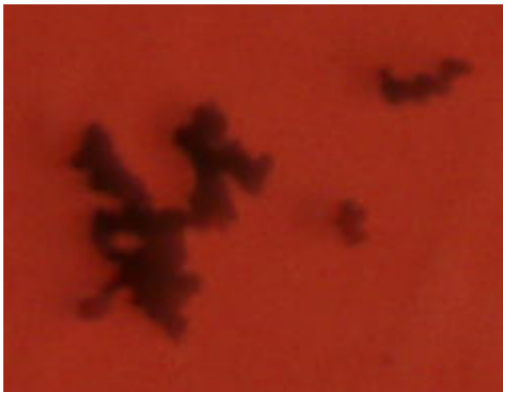

Figure 1. *Karyotype of Tabanus autumnalis (Szilády 1914) (7n= 14 chromosomes).*

The mitotic chromosomes were not spread properly in the preparation. Six pairs of autosomal chromosomes plus one pair of sex chromosomes (smallest).

Tabanus leleani (Austen 1920) (Sh-T41) has shown seven pairs of chromosomes from ovarian cells of females and testis of males (Figure 2).

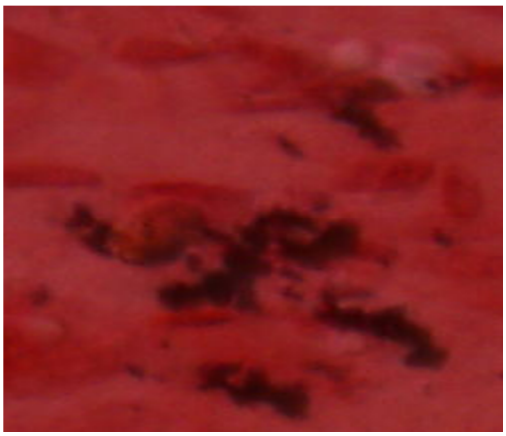

Figure 2. *Karyotype of Tabanus leleani (Austen 1920) (5n= 10 chromosomes).*

The mitotic chromosomes were not spread properly in the preparation. Four pairs of autosomal chromosomes plus one pair of sex chromosomes (smallest).

Atylotus pulchellus (Loew 1858) (Sh-T40) has shown six pairs of chromosomes from ovarian cells of females and testis of males (Figure 3).

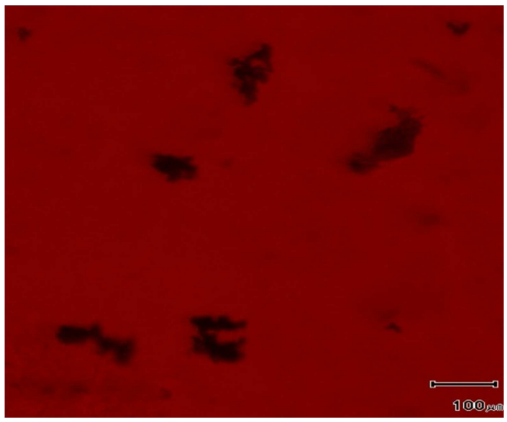

Figure 3. *Karyotype of Atylotus pulchellus (Loew 1858) (6n= 12 chromosomes).*

The mitotic chromosomes were not spread properly in the preparation. Five pairs of autosomal chromosomes plus one pair of sex chromosomes (smallest).

Hybomitra mendica (Villeneuve 1912) (Sh-T16) has shown six pairs of chromosomes from ovarian cells of females and testis of males (Figure 4).

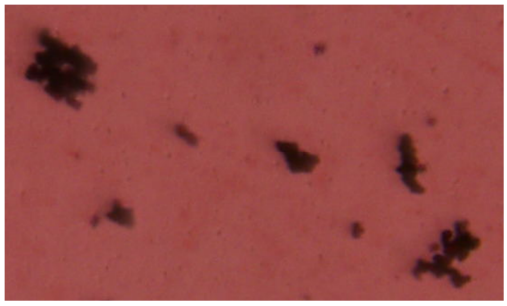

Figure 4. *Karyotype of Hybomitra mendica (Villeneuve 1912) (9n= 18 chromosomes).*

The mitotic chromosomes were not spread properly in the preparation. Six pairs of autosomal chromosomes plus one pair of sex chromosomes (smallest).

4. Discussion

Horse flies are an annoyance to livestock, as well as humans. Heavy biting rates can lead to weight loss in beef cattle, reduced milk yield in dairy cattle and hide damage from the fly's puncture wounds. Also investigators have isolated many viruses, bacteria, and protozoa from the sponge-like female mouthparts and their digestive system, but no studies to date show conclusive evidence that horse flies are capable of transmitting diseases to humans.

Comparative karyotype analysis has some advantages over other methods used in taxonomic studies of insects and other animals. In particular, chromosomal characters are basically morphological, and therefore, they can be analyzed approximately in the same way as other morphological features. In addition, methods of chromosomal analysis are

relatively inexpensive and allow an enormous material to be examined in a short time.

The current study reports the karyotypes of *Tabanus leleani* (Austen 1920) and *Hybomitra mendica* (Villeneuve 1912), for the first time. Previous studies have determined the diploid number of chromosomes of Tabanidae species ranged from 8 to 18 [6, 15]. This study, reports that *T. autumnalis* (Linnaeus 1761) has 2n= 14, *T. leleani* (Austen 1920) has 2n= 10, *Atylotus pulchellus* (Loew 1858) has 2n= 12, and *Hybomitra mendica* (Villeneuve 1912) has 2n= 18. The chromosome numbers of these species also fell within the reported range found in the literature (Table 2).

Tabanidae are divided in to three subfamilies, Pangoninae, Chrysopsinae, and Tabaninae. No study has focused on the cytotaxonomic properties of Pangoninae. According to Ivanischuk (1986), chromosome number of Chrysops species belonging to Chrysopsinae range from 8 to 12; [6] Tabaninae species from 10 to 18 and these are consistent with the present study. However, a clear difference in the chromosomes numbers between these two families is observed.

The considerable chromosomal variability observed in the genus *Tabanus* (Table 1) indicates that chromosomal changes may have played a role in the speciation events in this genus. Also species richness of *Tabanus* genus, have more species than other genera and this may be due to changes in the chromosomal structure in *Tabanus* species which are more familiar to adaptation than other species [3]. Moreover, based on these results it has been assumed previously that species belonging to the same group in this genus will have the same chromosome number [3]. The same study has reported that all investigated Tabanidae females display (XX) and males (XY) sex chromosome system. But in some cases, the X and Y are nearly homomorphic and accordingly difficult to identify. They are apparently the shortest pair in most species but varyc onsiderably in morphology in different species. In spite of a considerable reduction in diploid numbers, the XX: XY sex chromosome system is preserved in all horse fly species. It can be regarded as the ancestral system in horse flies, so same results were interpreted in many cytogenetical studies [31, 3].

Although karyotype variation in Tabanidae may give little information of phylogenetic significance, it will be a useful taxonomic tool for species identification. Similar studies should be carried out for other species of Tabanidae from different geographical regions of the Sudan to deduce relationships among species, and its evolutionary features can then be evaluated. Moreover, similar studies can be performed on different Dipteran families.

Table 2. *Chromosome numbers of some Tabanidae species.*

Species	Chromosome Number	Literature	Species	Chromosome Number	Literature
Tabanus autumnalis	14	Altunsoy & yavuz Kilic, 2010; Ivanischuk 1986	C. Indus	10	Boyes & Wilkes, 1972
T. bifarius	16	Boyes & Wilkes, 1972	C. ludens	10	Ivanischuk, 1986
T. bromius	10	Altunsoy & yavuz Kilic, 2010; Ivanischuk, 1986	C. mlokosiewiczi	10	Ivanischuk, 1986
T. flavofemoratus	10	Ivanischuk, 1986	C. pictus	10	Ivanischuk, 1986
T. bovines	14	Ivanischuk, 1986	C. relictus	10	Ivanischuk, 1986
T. buddha	12	Ivanischuk, 1986	C. suavis	10	Ivanischuk, 1986
T. colchidicus	16	Ivanischuk, 1986	C. shermani	8	Boyes & Wilkes, 1972
T. cordiger	12	Boyes & Wilkes; 1972; Ivanischuk, 1986	C. vanderwulpi	10	Ivanischuk, 1986
T. dolini	12	Ivanischuk, 1986	C. vittatus	12	Ivanischuk, 1986
T. geminus	14	Ivanischuk, 1986	Haematopotacrassicornis	18	Ivanischuk, 1986
T. hauseri	14	Ivanischuk, 1986	H. italica	14	Altunsoy & yavuz Kilic, 2010
T. indrae	10	Ivanischuk, 1986	H. pallens	18	Boyes & Wilkes, 1972; Ivanischuk, 1986
T. infestus	10	Ivanischuk, 1986	H. scutellatarossica	18	Ivanischuk, 1986
T. maculicornis	10	Ivanischuk, 1986	H. subcylindrica	26	Ivanischuk, 1986
T. marginalis	10	Boyes & Wilkes, 1972	H. tamerlani	18	Ivanischuk, 1986
T. miki	10	Ivanischuk, 1986	Hybomitralasiophthalma	14	Altunsoy & yavuzKilic, 2010
T. pleskei	12	Ivanischuk, 1986	H. arpadi	12	Ivanischuk, 1986
T. sabuletorum	12	Ivanischuk, 1986	H. bimaculata	14	Ivanischuk, 1986
T. spectabilis	10	Ivanischuk, 1986	H. brevis	18	Ivanischuk, 1986
T. sudeticus	14	Altunsoy & yavuzKilic, 2010	H. ciureai	12	Ivanischuk, 1986
T. shelkovnikovi	12	Ivanischuk, 1986	H. distinguendadistinguenda	16	Ivanischuk, 1986
T. quatuornotatus	16	Altunsoy & yavuzKilic, 2010	H. erberi	10	Ivanischuk, 1986
T. unifasciatus	12	Altunsoy & yavuzKilic, 2010; Ivanischuk, 1986	H. lundbecki	18	Ivanischuk, 1986
T. zimini	12	Ivanischuk, 1986	H. montanamontana	16	Ivanischuk, 1986
Atylotusfulvus	18	Altunsoy & yavuz Kilic, 2010; Boyes & Wilkes, 1972;	H. m. acrocentrica	16	Ivanischuk, 1986

Species	Chromosome Number	Literature	Species	Chromosome Number	Literature
		Ivanischuk, 1986			
A. bicolor	18	Boyes & Wilkes, 1972	H. muhlfeldi	10	Ivanischuk, 1986
A. horvathi	12	Ivanischuk, 1986	H. nigella	18	Ivanischuk, 1986
A. loewianus	18	Altunsoy & yavuz Kilic, 2010	H. peculiaris	14	Ivanischuk, 1986
A. obioensis	18	Boyes & Wilkes, 1972	H. stenopselapha	18	Ivanischuk, 1986
A. pulchelluskarybenthinus	18	Ivanischuk, 1986	H. tarandina	18	Ivanischuk, 1986
Chrysopsaberrans	12	Ivanischuk, 1986	H. tarandinoides	10	Ivanischuk, 1986
C. caecutiens	10	Ivanischuk, 1986	H. ussuriensis	18	Ivanischuk, 1986
C. flavipes	10	Ivanischuk, 1986	Dasyrhamphisumbrinus	12	Altunsoy & yavuz Kilic, 2010
C. frigidus	10	Boyes & Wilkes, 1972			

In all dipteran species and most cel ltypes studied to date, there is intimate somatic pairing from early prophase up to metaphase [22]. This somatic pairing is also observed in the karyotypes described in this study. The karyotype of most of the dipteran insects as consisting of 5 pairs of autosomal chromosomes and one pair of heterochromatic sex chromosome is well documented by several studies on Dung fly [30].

The high diversity in karyotype pattern of horse flies obtained by this study was in agreement with the previous statement that diversity within the Tabanidae is greatest in the tropics, but moist temperate regions have a rich fauna as well [26]. Moreover, this study showed the presence of two genetically differentiated populations of *Tabanus* species as sympatric in Gedaref region. This karyotypic information will help in understanding the inheritance of the phenotypic variation within the horse flies.

5. Conclusions

The present study reports the karyotypic pattern for the horse flies in Sudan from Al-Showak district in Gedaref state, for the first time and it revealed the presence of 5 patterns of karyotypes from Tabanidae namely; *Tabanus autumnalis* (Szilády 1914) 7n= 14; *Tabanus leleani* (Austen 1920) 5n= 10; *Atylotus pulchellus* (Loew 1858) 6n= 12; *Hybomitra mendica* (Villeneuve 1912) 9n= 18 and *Tabanus sp.* 8n= 16.

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