

Orijinal araştırma (Original article)

Karyotype characterization of some Tabanidae (Diptera) species¹

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Summary

Karyotypic features of Tabanidae (Diptera: Insecta) are largely unexplored. In this study, karyotypes of some horse fly species, *Tabanus quatuornotatus* Meigen, 1820, *T. sudeticus* Zeller, 1842, *Dasyrhamphis umbrinus* (Meigen, 1820), *Atylotus loewianus* (Villeneuve, 1920) and *Haematopota italica* Meigen, 1804 are described for the first time, and also, karyotypes of *T. autumnalis* Linnaeus, 1761, *T. bromius* (Linnaeus, 1761), *T. bifarius* Loew, 1858, *T. unifasciatus* (Loew, 1858), *A. fulvus* (Meigen, 1820) are confirmed. Karyotypic differences between six genera in Tabanidae will be discussed. Adult and larval specimens were collected, injected cholchicine and brought to the laboratory. After 24-48 hours tissues were dissected, fixed and dyed, respectively. After dying, preparations were made by squashing tissues and investigated with light microscope and photographed. Based on the results of the present study, the chromosome number in the following reads as follows; *T. autumnalis* 2n=14, *T. bromius* 2n=10, *T. bifarius* 2n=16, *T. quatuornotatus* 2n=16, *T. sudeticus* 2n=14, *T. unifasciatus* 2n=12, *D. umbrinus* 2n=10, *A. fulvus* 2n=18, *A. loewianus* 2n=18 and *H. italica* 2n=14 chromosome number. Chromosome number, chromosome arm length and arm ratio, location of centromere of these species is reported and evaluated.

Key words: Horse flies, Tabanidae, Diptera, Insecta, karyotype analysis

Anahtar sözcükler: At sinekleri, Tabanidae, Diptera, Insecta, karyotip analizi

Introduction

Females of most Tabanidae species attack mammals, principally Equidae, Bovidae, Camelidae and humans. Like other blood-sucking flies, tabanids have a negative economic effect on milk production where they commonly live (Chvala et al, 1972). Moreover, tabanids are known worldwide as important mechanical vectors of virus, bacteria, protozoans and helminthes,

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which cause diseases in some wild and domestic animals (Crosskey & Crosskey, 1955; Krinsky, 1976; Chvala & Jezek, 1997; Barros, 2001; Ferreira et al., 2002).

Applications of chromosome analysis are expanding because of the advantages of karyotypic analysis as taxonomic characters. This is true, in particular, of economically important groups of insects, including agricultural and forest pests (Blackman, 1985; Blackman et al., 1990; Fedorova et al., 1991; Maltempo & Avancini, 2000; 2001; Cook et al., 2002; Gokhman, 2004; Chubareva & Petrova, 2006), Gokhman & Kuznetsova, 2006; disease vectors (Coluzzi et al., 2002), and entomophages which control pest populations (Gokhman, 2006). However, limited studies concerning about chromosomal peculiarities of horse flies are found in the literature.

The family horse fly, one of the largest families of Diptera is divided into 8 genera with more than 650 described species in the Palearctic Region (Leclercq, 1985; Chvala, 1988). From a karyological point of view, it remains a poorly investigated group. Karyotypes of 64 species belonging to 5 genus have been published time the present (Boyes & Wilkes, 1972; Ivanischuk, 1983; 1986). Boyes & Wilkes (1972) carried out their studies in the Nearctic Region (Canada). Thus, in this location, we can easily argue that most of their analyses are not useful for identification and definition of species in the Palearctic Region.

In this study, chromosome number, chromosome arm length and arm ratio and location of centromere of ten species of Tabanidae were analyzed, and the results were compared with other species, previously analyzed by Boyes & Wilkes (1972), Ivanischuk (1983; 1986).

Material and Methods

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 this particular 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 (Chvala et al., 1972; Rimma, 1993; Chvala & Jezek, 1997; Squitier, 1998). Generally, in this study, mostly adult females were captured over the different horses in different habitats and some larvae collected from moist soils during the activity period incorporating the years 2005 and 2006.

Many different methods were tested for karyotype analysis, which were published in the literature up to present day (Ivanischuk, 1986; Rao & Rai, 1987; Hota & Patnatik, 1989; Warchalowska-Sliwa & Bugrov, 1996; De Prins et al., 2002a, 2002b, Rodriguez et al., 2002). Furthermore, an ideal working protocol for Tabanidae was developed and used. When specimens over horses, were secured, about 1µl (one or two drop) 0.4% - 0.5% colchicine

solution was injected promptly, from the abdominal region of fly with micro injector to achieve the cell divisions. Next, specimens were transferred to the laboratory with fly cage and waited for three-four hours period at room temperature. Later, ovarian tissue of females, testis of males and brain tissue of larva were dissected carefully in hypotonic sodium citrate solution (1% in dH₂O) in room temperature and transferred in carnoy fixative (3:1 Ethanol 70% : Glacial acetic acid 45%). These were deposited in this solution for 24 hours at a temperature - 20 °C. After fixation, tissues were washed with dH₂O and relocated in 0,1N HCl, 20 °C. Tissues were washed again with dH₂O and a suspension was prepared in a one drop of 2 % aceto-orcein or aceto-carmin on a microscope slide for a period 10 minutes for staining purpose. Then, coverglass was closed and sealed with entellan or colorless nail polish. Metaphase plates were observed by an Olympus (M51) research microscope.

To obtain data on chromosome morphology, ten plates of a mitotic metaphase stage were measured and evaluated for each species. Relative chromosome lengths were calculated as a percentage of the total chromosome length of the haploid set (TCL), including the sex chromosome. Chromosome morphologies were classified according to Levan et al. (1964).

Results

Dasyrhamphis umbrinus (Meigen, 1820)

Six pairs of chromosomes from ovarian cells of adult *Dasyrhamphis umbrinus* (Meigen, 1820) males were secured (Fig.1). Arm lengths of these six complements were measured between 58.6µ and 68.4µ and relative TCL (Total Complement Length) was calculated as 64.3µ (±2µ) (Table 1).

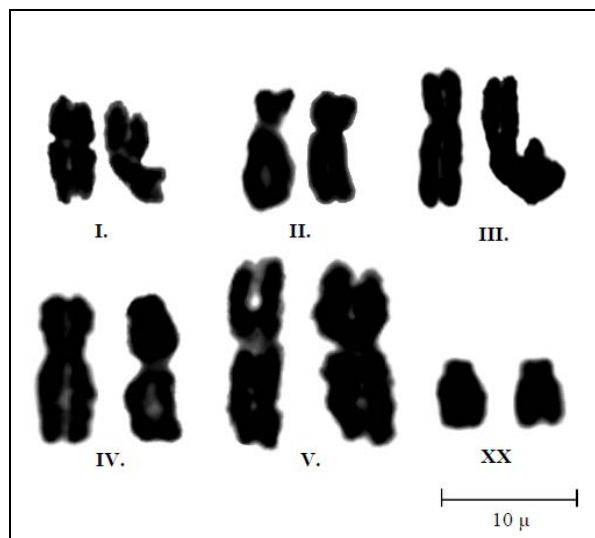


Figure 1. Karyotype of *Dasyrhamphis umbrinus* (Meigen, 1820).

Table 1. Chromosomal peculiarities of *Dasyrhamphis umbrinus* (Meigen, 1820) (m=Metacentric, sm= Submetacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	XX
Relative Arm Lengths (μ)	8.6	9.4	12.3	13.4	14.9	5.8
Percentages in TCL (%)	13.4	14.3	19.1	20.8	23.3	9.1
Arm Ratios	1.01	2.09	1.25	1.03	1.06	1.17
Chromosome morphology	m	sm	m	m	m	m

Haematopota italica (Meigen, 1804)

Seven pairs of chromosomes from ovarian cells of adult females and brain cells of larvae were established (Fig. 2). Arm lengths of these eighth complements were measured between 49.2 μ with 65.3 μ and relative TCL was calculated as 52.4 μ (\pm 2 μ) (Table 2).

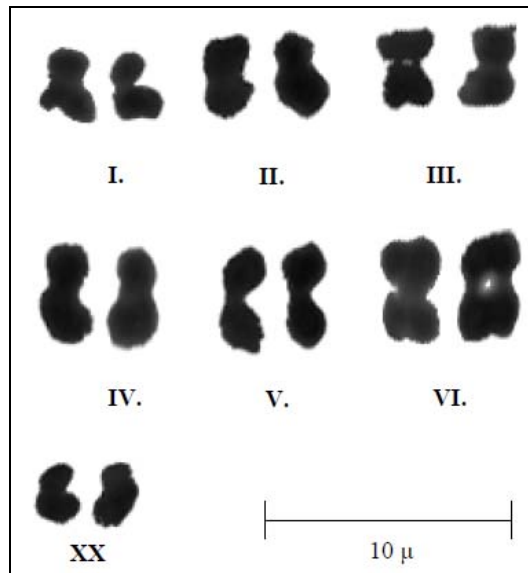


Figure 2. Chromosomal peculiarities of *Haematopota italica* (Meigen, 1804).

Table 2. Chromosomal peculiarities of *Haematopota italica* (Meigen, 1804) (m=Metacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	XX
Relative Arm Lengths (μ)	6.5	7.4	7.9	8.1	8.7	9.6	4.2
Percentages in TCL (%)	12.4	14.1	15.0	15.5	16.7	18.3	8.01
Arm Ratios	1.20	1.43	1.39	1.53	1.18	1.05	1.25
Chromosome morphology	m	m	m	m	m	m	m

Atylotus fulvus (Meigen, 1820)

The male karyotype of *Atylotus fulvus* (Meigen, 1820) comprises 18 chromosomes, 8 autosome pairs and one pair X chromosomes (Fig. 3). Arm lengths of these nine complements were measured between 72.5 μ with 79.2 μ and relative TCL was calculated as 75.3 μ ($\pm 2\mu$) (Table 3).

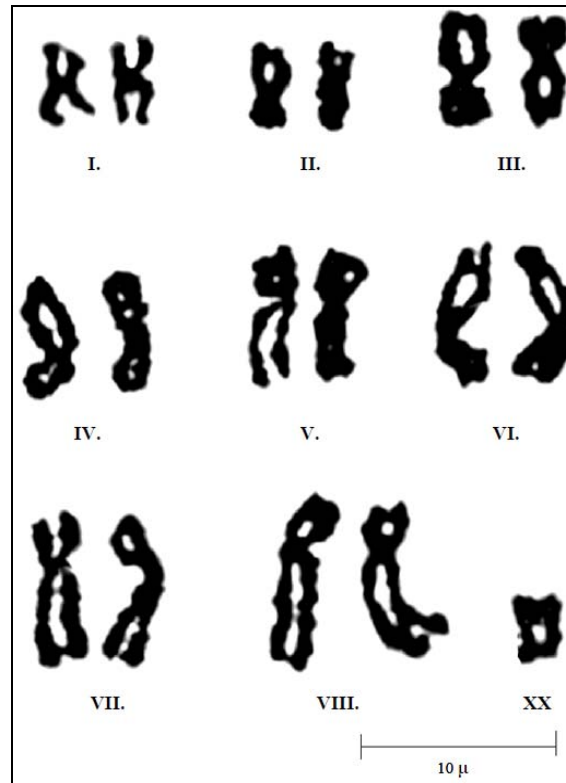


Figure 3. Chromosomal peculiarities of *Atylotus fulvus* (Meigen, 1820).

Table 3. Chromosomal peculiarities of *Atylotus fulvus* (Meigen, 1820) (m=Metacentric, sm= Submetacentric, sa=Subacrocentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	XX
Relative Arm Lengths (μ)	5.1	6.0	7.7	8.4	9.2	10.6	12.7	13.2	4.1
Percentages in TCL (%)	6.70	7.86	10.05	10.95	11.72	13.66	16.62	17.23	5.41
Arm Ratios	1.18	1.95	13.3	1.24	1.89	1.21	1.92	4.25	2.90
Chromosome morphology	m	sm	sm	m	sm	m	sm	sa	sm

Atylotus loewianus (Villeneuve, 1920)

Nine pairs of chromosomes from ovarian cells of adult females were perceived (Fig. 4). Arm lengths of these nine complements were measured

between 74.3 μ with 81.0 μ and relative TCL was calculated as 78.6 μ ($\pm 2\mu$) (Table 4).

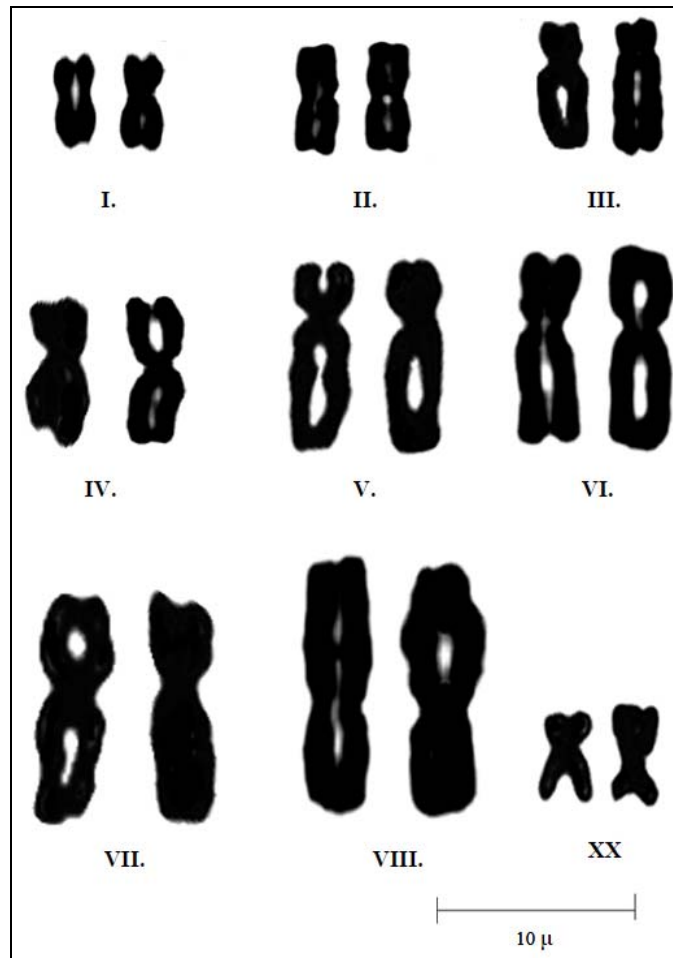


Figure 4. Karyotype of *Atylotus loewianus* (Villeneuve, 1920).

Table 4. Chromosomal peculiarities of *Atylotus loewianus* (Villeneuve, 1920) (m=Metacentric, sm= Submetacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	XX
Relative Arm Lengths (μ)	5.5	6.5	6.8	8.2	9.0	10.5	12.8	14.0	4.8
Percentages in TCL (%)	6.8	7.7	9.7	10.9	11.2	13.8	16.9	17.8	5.2
Arm Ratios	1.08	1.35	1.98	1.16	2.01	1.52	1.40	1.20	2.03
Chromosome morphology	m	m	sm	m	sm	m	m	m	sm

***Tabanus autumnalis* Linnaeus, 1761**

Seven pairs of chromosomes from ovarian cells of adult females were obtained (Fig. 5). Arm lengths of these seven complements were measured between 65.2 μ with 76.8 μ and relative TCL was calculated as 68.9 μ ($\pm 2\mu$) (Table 5).

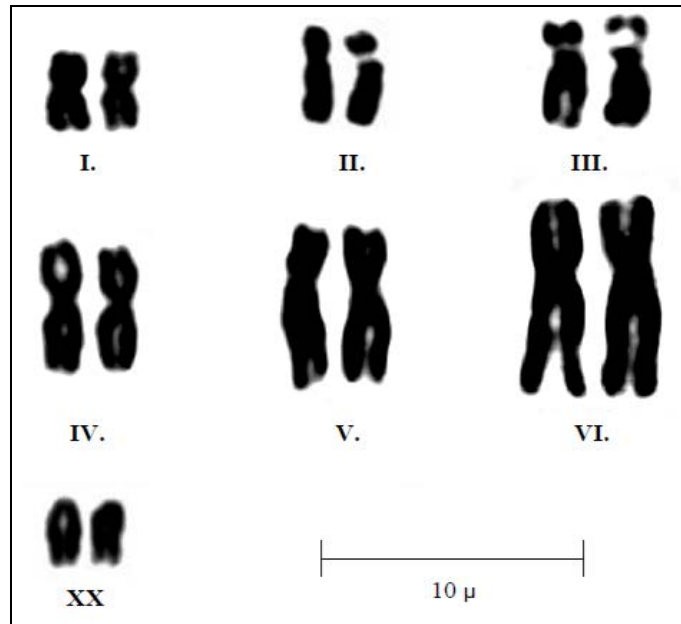


Figure 5. Karyotype of *Tabanus autumnalis* Linnaeus, 1761.

Table 5. Chromosomal peculiarities of *Tabanus autumnalis* (m=Metacentric, sm= Submetacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	XX
Relative Arm Lengths (μ)	7.4	8.5	9.7	10.8	12.8	15.0	4.7
Percentages in TCL (%)	10.7	12.3	14.1	15.7	18.6	21.8	6.8
Arm Ratios	1.15	2.21	2.03	1.17	1.98	1.23	1.32
Chromosome morphology	m	sm	sm	m	sm	m	m

***Tabanus bifarius* Loew, 1858**

Eight pairs of chromosomes from ovarian cells of adult females and larval brain cells were deduced (Fig. 6). Arm lengths of these complements were measured between 65.2 μ with 76.8 μ and relative TCL was calculated as 68.9 μ ($\pm 2\mu$) (Table 6).

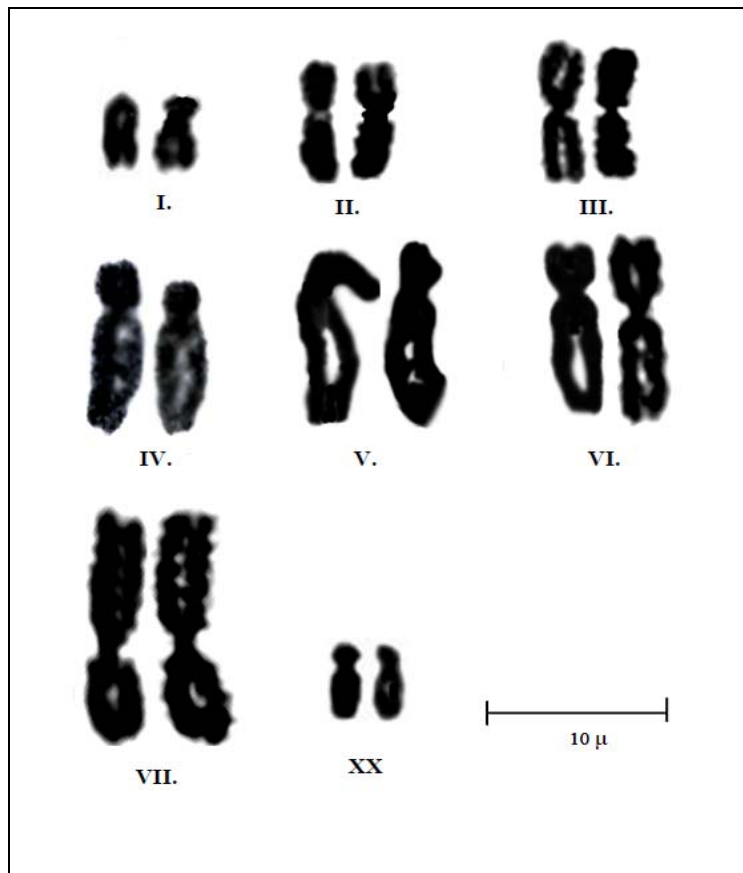


Figure 6. Karyotype of *Tabanus bifarius* Loew, 1858.

Table 6. Chromosomal peculiarities of *Tabanus bifarius* Loew, 1858 (m=Metacentric, sm= Submetacentric, sa=Subacrocentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	XX
Relative Arm Lengths (μ)	5.9	6.5	7.9	8.5	10.5	11.3	11.9	4.2
Percentages in TCL (%)	8.85	9.75	11.85	12.74	15.74	16.94	17.84	6.30
Arm Ratios	3.28	2.03	1.52	1.98	2.45	2.16	2.75	1.18
Chromosome morphology	sa	sm	m	sm	sm	sm	sm	m

Tabanus bromius (Linnaeus, 1761)

Five pairs of chromosomes from ovarian cells of adult females, egg cells of these females and testis cells of one male were recognized (Fig. 7). Arm lengths of these six complements were measured between 52.4μ with 76.8μ and relative TCL was calculated as 68.5 μ (± 3μ) (Table 7).

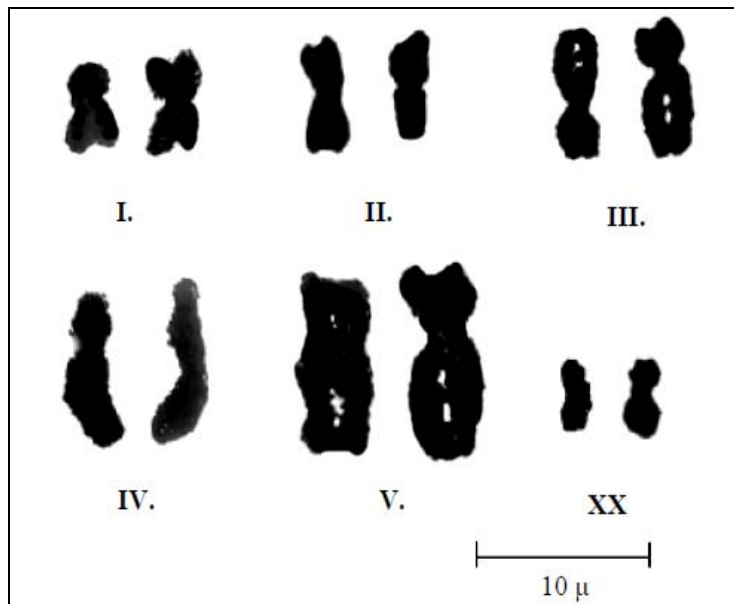


Figure 7. Karyotype of *Tabanus bromius* (Linnaeus, 1761).

Table 7. Chromosomal peculiarities of *Tabanus bromius* (Linnaeus, 1761) (m=Metacentric, sm= Submetacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	XX
Relative Arm Lengths (μ)	9.7	11.5	13.5	15.2	4.6
Percentages in TCL (%)	15.6	18.4	21.6	24.7	7.6
Arm Ratios	2.21	2.03	1.17	1.98	1.23
Chromosome morphology	sm	sm	m	m	m

***Tabanus quatuornotatus* Meigen, 1820**

There have been obtained six pairs of chromosomes from ovarian cells of adult females and brain cells of two larvae were unfolded (Fig. 8). Arm lengths of these six complements were measured between 67.8 μ with 79.8 μ and relative TCL was calculated 75.3 μ ($\pm 3\mu$) (Table 8).

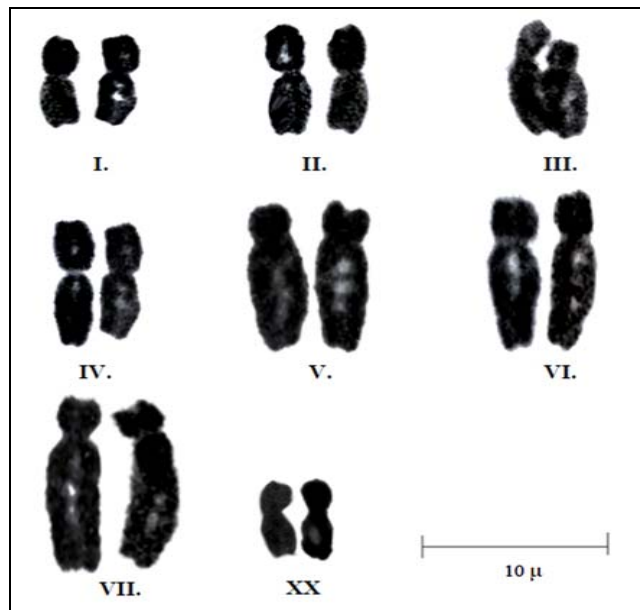


Figure 8. Karyotype of *Tabanus quatuornotatus* Meigen, 1820.

Table 8. Chromosomal peculiarities of *Tabanus quatuornotatus* Meigen, 1820 (m=Metacentric, sm= Submetacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	XX
Relative Arm Lengths (μ)	6.4	7.7	9.1	9.8	11.2	12.0	13.8	4.9
Percentages in TCL (%)	6.5	9.4	11.4	12.8	14.0	15.2	17.1	5.2
Arm Ratios	1.20	1.09	2.01	1.52	2.34	2.04	1.87	1.16
Chromosome morphology	m	m	sm	m	sm	sm	sm	m

***Tabanus sudeticus* Zeller, 1842**

Seven pairs of chromosomes from ovarian cells of adult females and testes of one male were established (Fig. 9). Arm lengths of these seven complements were measured between 63.2 μ with 71.4 μ and relative TCL was calculated as 66.9 μ (± 2μ) (Table 9).

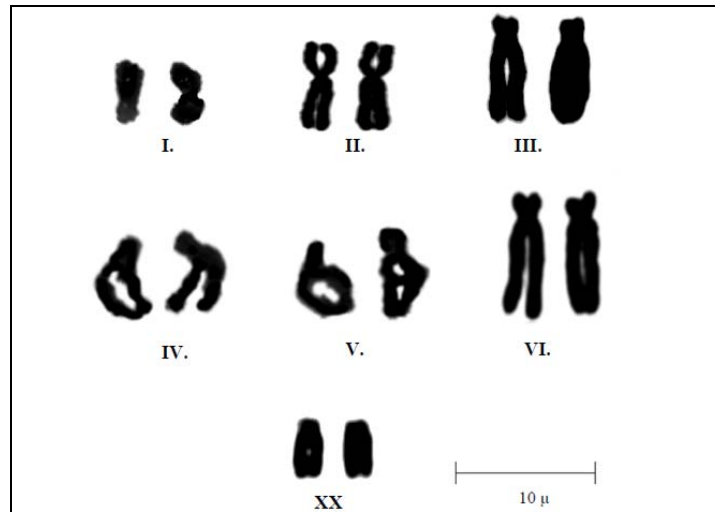


Figure 9. Karyotype of *Tabanus sudeticus* Zeller, 1842.

Table 9. Chromosomal peculiarities of *Tabanus sudeticus* Zeller, 1842 (m=Metacentric, sm= Submetacentric, sa=Subacrocentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	6 th	XX
Relative Arm Lengths (μ)	6.1	8.4	10.0	11.3	12.5	13.5	5.1
Percentages in TCL (%)	9.2	12.6	14.9	16.9	18.6	20.2	7.6
Arm Ratios	1.35	1.08	2.21	1.95	4.20	3.36	1.01
Chromosome morphology	m	m	sm	sm	sa	sa	m

***Tabanus unifasciatus* (Loew, 1858)**

Six pairs of chromosomes from ovarian cells of adult females and brain cells of two larvae were decoded (Fig. 10). Arm lengths of these six complements were measured between 49.2μ with 65.3μ and relative TCL was calculated as 56.2 μ (± 2μ) (Table 10).

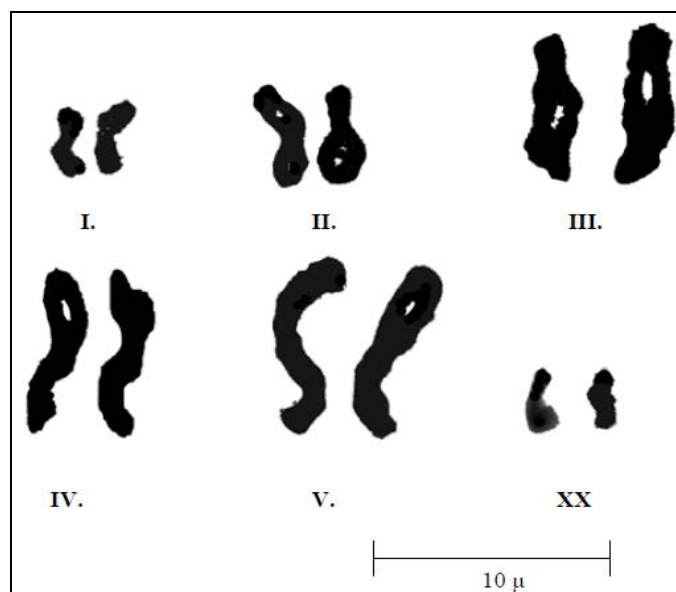


Figure 10. Karyotype of *Tabanus unifasciatus* (Loew, 1858).

Table 10. Chromosomal peculiarities of *Tabanus unifasciatus* (Loew, 1858) (m=Metacentric, sm= Submetacentric)

Chromosome Pairs	1 st	2 nd	3 rd	4 th	5 th	XX
Relative Arm Lengths (μ)	6.1	8.4	10.0	11.3	12.5	4.4
Percentages in TCL (%)	9.4	11.4	15.2	17.1	18.4	8.01
Arm Ratios	1.39	1.05	2.40	1.25	2.22	1.18
Chromosome morphology	m	M	sm	m	sm	m

Discussion

Comparative karyology has some advantages over other methods used in taxonomic studies of insects and other animals. In particular, chromosomal characters are essentially morphological and therefore, they can be analyzed approximately in the same way as other morphological features. Moreover, methods of chromosomal analysis are relatively inexpensive and allow vast material to be examined in a short space of time.

Conversely, unlike other studies, this study reports karyotypes of *Tabanus quatuornotatus* Meigen, 1820, *Tabanus sudeticus* Zeller, 1842, *Dasyrhamphis umbrinus* (Meigen, 1820), *Atylotus loewianus* (Villeneuve, 1920) and *Haematopota italica* Meigen, 1804, for the first time. Information about chromosome structure of *Dasrhamphis* genus has yet been discussed. It was determined in previous studies that the diploid number of chromosomes of Tabanidae species ranged from 8 to 18 (Boyes & Wilkes, 1972; Ivanischuk, 1983; 1986). In this study, It emerged that *T. autumnalis* Linnaeus, 1761 has $2n=14$, *T. bromius* (Linnaeus,

1761) has $2n=10$, *T. bifarius* Loew, 1858 has $2n=16$, *T. quatuornotatus* has $2n=16$, *T. sudeticus*, 1842 has $2n=14$, *T. unifasciatus* (Loew, 1842) has $2n=12$, *D. umbrinus* has $2n=10$, *A. fulvus* has (Meigen, 1820) $2n=18$ *A. loewianus* $2n=18$ and *H. italica* has $2n=14$. The chromosome numbers of these species also fell within the reported range found in the literature (Table 11).

Tabanidae are divided in three subfamilies, Pangoninae, Chrysopsinae and Tabaninae. Up to present day, no study has focused on the cytotaxonomical properties of Pangoninae. According to Boyes & Wilkes (1972) chromosome number of *Chrysops* species belonging to Chrysopsinae range from 8 to 12 and are consistent with the present study, Boyes & Wilkes (1972) Tabaninae species from 10 to 18. In this instance, clear differences in terms of chromosome number between these two families were observed.

Table 11. Chromosome numbers of some Tabanidae species

Species	Chromosome Number	Literature
<i>Tabanus autumnalis</i>	14	This study; Ivanischuk 1986
<i>T. bifarius</i>	16	Boyes & Wilkes, 1972
<i>T. bromius</i>	10	This study; Ivanischuk, 1986
<i>T. flavofemoratus</i>	10	
<i>T. bovines</i>	14	Ivanischuk, 1986
<i>T. buddha</i>	12	
<i>T. colchidicus</i>	16	
<i>T. cordiger</i>	12	Boyes & Wilkes; 1972; Ivanischuk, 1986
<i>T. dolini</i>	12	
<i>T. geminus</i>	14	
<i>T. hauseri</i>	14	Ivanischuk, 1986
<i>T. indrae</i>	10	
<i>T. infestus</i>	10	
<i>T. maculicornis</i>	10	
<i>T. marginalis</i>	10	Boyes & Wilkes, 1972
<i>T. miki</i>	10	
<i>T. pleskei</i>	12	Ivanischuk, 1986
<i>T. sabuletorum</i>	12	
<i>T. spectabilis</i>	10	
<i>T. sudeticus</i>	14	This study
<i>T. shelkovnikovi</i>	12	Ivanischuk, 1986
<i>T. quatuornotatus</i>	16	This study
<i>T. unifasciatus</i>	12	This study; Ivanischuk, 1986
<i>T. zimini</i>	12	Ivanischuk, 1986
<i>Atylotus fulvus</i>	18	This study; Boyes & Wilkes, 1972; Ivanischuk, 1986
<i>A. bicolor</i>	18	Boyes & Wilkes, 1972
<i>A. horvathi</i>	12	Ivanischuk, 1986
<i>A. loewianus</i>	18	This study
<i>A. obioensis</i>	18	Boyes & Wilkes, 1972

Table 11. Chromosome numbers of some Tabanidae species (Continue)

Species	Chromosome Number	Literature
<i>A. pulchellus karybenthinus</i>	18	
<i>Chrysops aberrans</i>	12	
<i>C. caecutiens</i>	10	Ivanischuk, 1986
<i>C. flavipes</i>	10	
<i>C. frigidus</i>	10	
<i>C. Indus</i>	10	Boyes & Wilkes, 1972
<i>C. ludens</i>	10	
<i>C. mlokosiewiczzi</i>	10	
<i>C. pictus</i>	10	Ivanischuk, 1986
<i>C. relictus</i>	10	
<i>C. suavis</i>	10	
<i>C. shermani</i>	8	Boyes & Wilkes, 1972
<i>C. vanderwulpi</i>	10	
<i>C. vittatus</i>	12	Ivanischuk, 1986
<i>Haematopota crassicornis</i>	18	
<i>H. italica</i>	14	This study
<i>H. pallens</i>	18	Ivanischuk, 1986
<i>H. pellucens</i>	14	
<i>H. pluvialis</i>	18	Boyes & Wilkes, 1972; Ivanischuk, 1986
<i>H. scutellata rossica</i>	18	
<i>H. subcylindrica</i>	26	Ivanischuk, 1986
<i>H. tamerlani</i>	18	
<i>Hybomitra lasiophthalma</i>	14	This study
<i>H. arpadi</i>	12	
<i>H. bimaculata</i>	14	
<i>H. brevis</i>	18	
<i>H. ciureai</i>	12	
<i>H. distinguenda distinguenda</i>	16	
<i>H. erberi</i>	10	
<i>H. lundbecki</i>	18	
<i>H. montana montana</i>	16	Ivanischuk, 1986
<i>H. m. acrocentrica</i>	16	
<i>H. muhlfeldi</i>	10	
<i>H. nigella</i>	18	
<i>H. peculiaris</i>	14	
<i>H. stenopselapha</i>	18	
<i>H. tarandina</i>	18	
<i>H. tarandinoides</i>	10	
<i>H. ussuriensis</i>	18	
<i>Dasyrhamphis umbrinus</i>	12	This study

The considerable chromosomal variability observed in the genus *Tabanus* (Table 11) indicates that chromosomal changes may have played an important role in the speciation events in this genus. On the other hand, species richness of *Tabanus* genus, have more species than other genera. This may be related with changes in the chromosomal structure and *Tabanus* species which are more accustomed to adaptation than other species. Conversely, based on these results we can easily argue that species belonging to the same group in this genus,

secure the same chromosome number. Bromius group have $2n=10$, Bovinus group have $2n=14$ except *T. spectabilis*, Cordiger group have $2n=12$, Quatuornotatus group have $2n=16$ chromosome number.

In Tabanidae, most of the karyotype variation seems to involve the autosomes. Species with autosomes differing in number and relative length mostly have similar X chromosomes. Where these could be identified, they contributed 5-9 % of the total chromosome length. X chromosomes are much smaller in *Atylotus* and the longest in *Dasyrhamphis* genus.

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 vary considerably 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 study (Stahlavsky et al., 2006).

Although karyotype variation in Tabanidae may yield little information of phylogenetic significance, it will be a useful taxonomic tool for species recognition. It is apparent that the family is quite karyotypically variable and these results supported by karyological peculiarities may be useful for identification and definition of Tabanidae species.

It's thought that, if similar studies carry out for other species of Tabanidae, relationships among species and its evolutionary features may be evaluated. Moreover, the protocol used in this study may be useful for other studies related to the different Dipteran families.

Özet

Bazı Tabanidae (Diptera) türlerinin karyotip karakterizasyonu

Tabanidae (Diptera: Insecta) türlerinin karyotip özellikleri büyük oranda araştırılmamıştır. Bu çalışmada bazı Tabanidae türlerinin, *Tabanus quatuornotatus* Meigen, 1820, *T. sudeticus* Zeller 1842, *Dasyrhamphis umbrinus* (Meigen, 1820), *Atylotus loewianus* (Villeneuve, 1920) ve *Haematopota italica* Meigen, 1804, karyotipik özellikleri ilk kez açıklanırken, bazı türlerin karyotipik özellikleri *T. autumnalis* Linnaeus, 1761, *T. bromius* (Linnaeus, 1761), *T. bifarius* Loew, 1858, *T. unifasciatus* (Loew, 1858), *A. fulvus* (Meigen, 1820), doğrulanmıştır. Tabanidae familyasına ait altı cinsin karyotipik farklılıkları tartışılmıştır. Ergin ve larval örnekler yakalandıktan sonra kolşisin enjekte edilmiş ve laboratuara getirilmiştir. 24-48 saat sonra dokular çıkarılarak fikse edilmiş ve boyanmıştır. Boyama sonrası dokular ezilerek preparat hazırlanmış ve mikroskopta inceleme yapılmış ve fotoğrafları çekilmiştir. Çalışma sonuçlarına göre; *T. autumnalis* $2n=14$, *T. bromius* $2n=10$,

T. bifarius 2n=16, *T. quatuornotatus* 2n=16, *T. sudeticus* 2n=14, *T. unifasciatus* 2n=12, *D. umbrinus* 2n=10, *A. fulvus* 2n=18, *A. loewianus* 2n=18 ve *H. italica* 2n=14 kromozom sayına sahiptir. Bu türlerin kromozom sayıları, kromozom kol uzunlukları ve oranları, sentromer konumları kaydedilmiş ve değerlendirilmiştir.

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