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Research

TRsandflies: A Web-Based Software for the Morphometric Identification of Sand Flies in Turkey

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Abstract

Sand flies are vector of several diseases, mostly cutaneous and visceral leishmaniasis (CL and VL). Also, 29 sand fly species have been identified in previous fauna studies carried out in 40 provinces of Turkey. Totally, 24 sand flies species belonging to *Phlebotomus* (*Ph.*) (Diptera: Psychodidae) genus have been proven or reported as possible vector species. This study aimed to develop a new software which could contribute to researchers' decision making about the identification of sand flies with obtained data from entomological surveys conducted before in Turkey. Developed software called TRsandflies included 35 textbox created with parameters obtained from caught sand flies specimens by the above-mentioned surveys. It also contained 130 photos and distribution maps related to 24 sand flies species. In addition, C# language and MYSQL database were used in the program. TRsandflies had three different forms (pages) allowing the user to compare the specimens and known species. In the species identification trials with three repetitions carried out in the program, except for the specimens belonging to the *Transphlebotomus* Artemiev & Neronov, 1984 subgenus, morphometric data of all previously collected sand fly species specimens were included. The process of running the morphometric measurement results of predetermined specimens in the program provided us with an accurate prediction rate of 86.66% in male specimens and 71.66% in female specimens. We concluded that the web-based software developed could play an important role in reducing the rate of possible errors that might be encountered by conventional identification methods.

Key words: TRsandflies, sand flies, identification, C#, MYSQL

Approximately 800 sand flies species have been described throughout the World. Previously, researchers using several entomological surveys described ~375 sand fly species (Galati 2003), and sand flies belonging to *Phlebotomus* subgenus were accepted to be possible vectors in the Old World. Especially, they are commonly seen in countries having Mediterranean climate and sand fly vectors, usually *Larroussius* (Diptera: Psychodidae) subgenus, where CL and VL diseases caused by *Leishmania* (*L.*) donovani Ross, 1903 and *Larroussius infantum* Nicolle, 1908 (Kinetoplastida: Trypanosomatida) (Inceboz 2019). In addition, sand fly-borne phleboviruses are believed to be important agents of human meningoencephalitis in the above-mentioned foci. However, the vertebrates acting as reservoirs for them have not yet been determined (Dincer et al. 2015).

Sand flies are small insects whose length of females and males specimens are 1.2–3.7 mm. Among our specimens and data, *Phlebotomus neglectus* Tonnoir, 1921 and *Phlebotomus major* Yakimoff males had the biggest length of body with 3.13 mm average, whereas *Phlebotomus simici* Nitzulescu, 1931 was observed to be the smallest with 1.80 mm in Turkey. In addition, *Ph. neglectus* and *Ph. major* with 3.15 mm showed the biggest length of body, whereas *Ph. simici* with 2.00 mm was determined to be the smallest in Turkey (Perfil'ev 1968, Lewis 1982, Yaman and Dik 2006, Ramazani et al. 2013, Kavur et al. 2018).

The description of sand flies has been become an important factor to determine the distribution and control of diseases they transmit to human beings since the sand fly was first identified by Scopoli in 1786 as *Bibio papatasi*. The method based on morphometric measurements and various authors' identification keys (phlebotometry) is commonly conducted in the sand fly identification process. Conventionally, the researchers first measure the segments of the antennae and palps for making a formula. Second, they measure the epipharynx, the length and the breadth of the wing to formulate wing indices. Then, they determine/compare the ratio of the length of the third antennal segment to that of the epipharynx. They finally measure the head, the thorax, the abdomen, the segments of the hind legs (femur and tibia), and in males also the terminalia. This system was called phlebotometry (Perfil'ev 1968).

Currently, in addition to conventional methods, the modern methods based on DNA analysis and electronic/digital keys are used when the morphological measurements are not enough for identification (Karakülah et al. 2016). For an accurate identification, it

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is vital that comprehensible measurements of morphological characters and maybe DNA analyses be used. However, the identification of some specimen belonging to *Larrouisous* subgenus such as *Ph. neglectus* and *Phlebotomus syriacus* Adler & Theodor, which are often called sister species is more difficult than the identification of others. Therefore, specialist examinations is needed in the recognition of special species DNA-based analyzes only confirm the final decisions of specialists in discriminating sister species and damaged specimens identification (Alten et al. 2015).

There has been interactive/electronic/digital/interactive/web-based identification softwares of arthropods used worldwide by many researchers, such as female mosquitoes (Nicolasa et al. 2016), ants (Seltmann 2004), and greenbugs (Elliott et al. 2004). There were only two web-based identification tools for sand flies (Vignes-Lebbe and Gallut 1997, Niang et al. 2004). In addition, there are web-based decision support softwares for many subjects in the insect identification programs (Karakülah et al. 2016).

Recently, 29 sand fly species [5 *Sergentomyia* (Diptera: Psychodidae) species and 24 *Phlebotomus* species] have been recognized in Turkey. There are no data and information on the vector capacity of six sand fly species in Turkey. It has been proven that other species transmit leishmaniasis agents in laboratory and field studies worldwide (Ok et al. 2002, Akhoundi et al. 2016).

My present study focused on developing a web-based identification software that consisted of inquiry and filtering features for species belonging to the genus *Phlebotomus* in Turkey.

Materials and Methods

Data Collection and Identification

Sand fly specimens were collected from selected locations using CDC light traps in different periods. Traps were placed in animal (goat, cattle, sheep, or poultry) sheds, and they stayed there for one night (Alten et al. 2015). Specimens were kept in 96% alcohol for morphological identifications, which were based on the morphology of male genitalia and female spermatheca and pharynges using several written keys. Until recently, there have been many taxonomy and fauna studies including morphological measurements of 29 sand fly species conducted in about 40 provinces in Turkey. It has taken ~60 yr since the beginning of the identification and presentation of the findings. The findings of the aforementioned studies, including 20 sand fly species of medical importance, were combined with the findings obtained from field studies, I have conducted for 10 yr [Afyon (2009–2011), Nigde (2009–2011), Imamoglu-Adana (2013–2014), Karaisali-Adana (2015–2016), and Kozan-Adana (2016–2017)]. In addition, sand flies identification keys, illustrations and pictures used for conventional method were also considered (Perfil'ev 1968, Abonnenc 1972, Lewis 1982, Leger et al. 2000, Depaquit et al. 2005).

Creatining Database and Interface of TRsandflies

The data of the morphological measurements of sand flies that entered into Microsoft Excel program were imported into Microsoft SQL Server Management Studio. Table was created for the data on which TRsandflies would use.

The main display of the TRsandflies was its interface screen that users can access the parameters. In addition, the interface design was created in the 'Visual Studio Professional' compiler by coding with the programming language 'C#'. The algorithm of the software is based on comparing the values of most important 38 parameters (Table 1) (length of coxite, style, genital pump, etc., for male, length of pharynx, epipharynx, number of spermatheca segments for female specimens) with the measurement results that the user will enter into the program (Fig. 1). After the separation of the gender, TRsandflies' algorithm has

Table 1. Parameters used in the database of TRsandflies

No.	Type of parameters	Parameters of male specimens	Type of parameters	Parameters of female specimens
1	Qualitative	**Taxonomic information** • Genus • Subgenus • Species	Qualitative	**Taxonomic information** • Genus • Subgenus • Species
2	Qualitative	Distribution	Qualitative	Vectorial capacity
3	Quantitative	Length (mm)	Qualitative	Transmitted disease(s)
4	Quantitative	Wing length (mm)	Qualitative	Reservoir(s)
5	Quantitative	Wing breadth (mm	Qualitative	Distribution
6	Quantitative	Epipharynx length (mm)	Quantitative	Length (mm)
7	Quantitative	Third antennal segment Length (mm)	Quantitative	Wing length (mm)
8	Quantitative	Sum of the fourth and fifth Antennal Segment Length (mm)	Quantitative	Wing breadth (mm
9	Quantitative	Coxite length (mm)	Quantitative	Third antennal segment length (mm)
10	Quantitative	Style length (mm)	Quantitative	Sum of the fourth and fifth antennal segment length (mm)
11	Quantitative	Style spines number	Quantitative	Pharynx length (mm)
12	Qualitative	Basal lobe	Quantitative	Epipharynx length (mm)
13	Quantitative	Number of hairs on basal lobe	Quantitative	Rate of armature and pharynx
14	Quantitative	Genital filament length (mm)	Quantitative	Number of spermatheca segments
15	Quantitative	Genital pump length (mm)	Quantitative	Length of labrum (mm)
16	Qualitative	Aedeagus type	Qualitative	Antennal formula
17	Quantitative	Aedeagus length (mm)	Qualitative	Palpal formüla
18	Qualitative	Parameter type	–	–
19	Quantitative	Number of hairs on coxite	–	–
20	Qualitative	Antennal formula	–	–
21	Qualitative	Palpal formüla	–	–

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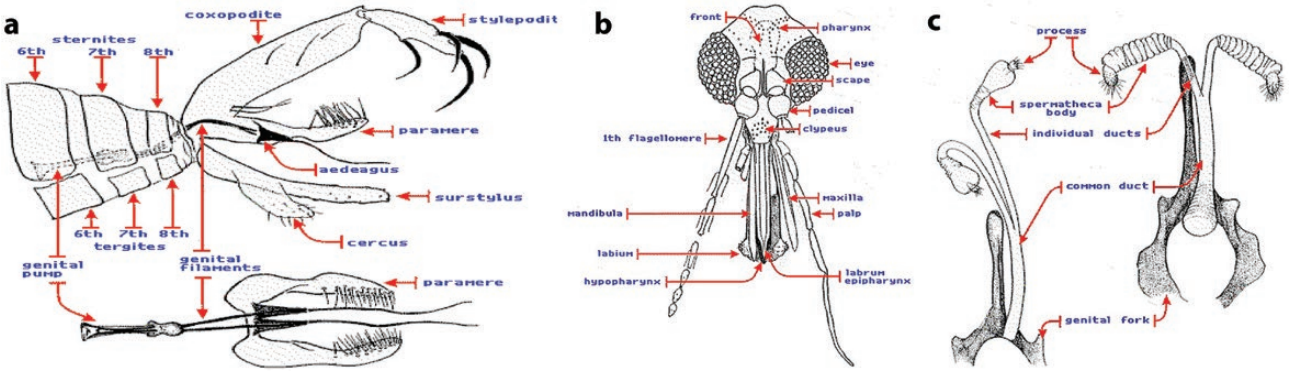


Fig. 1. The most significant morphological characters of sand flies: (a) genitalia of male specimens, (b) morphology of female head, and (c) genitalia of female specimens.

a flowchart that contains various morphological differences between species and responses to the questions related to these differences. In the interface of TRsandflies, the main query screen is designed as the screen where the user can enter the measurement values (gender, coxide length, style length, third antenna segment length, etc.). There are also the screens which reflect the suggestions for the decision of identification as a result of the comparison of the measurement values and screens which display the information about the related species.

The algorithm of TRsandflies is primarily based on the questioning of morphological measurements of male and female specimens of medically important five subspecies (*Phlebotomus*, *Paraphlebotomus* (Diptera: Psychodidae), *Larroussius*, *Transphlebotomus*, and *Adlerius* (Diptera: Psychodidae)) of sandflies in Turkey (Figs. 2 and 3). Although *Transphlebotomus* is included in the algorithm of the newly designed software, it could not be included in the database as no reliable data could be found.

Testing and Running the TRsandflies

The measurements of aforementioned characters of 20 sand fly specimens belonging to different subgenus previously identified by conventional methods were entered into the program three times by three students (expect for four *Transphlebotomus* species) in order to test the ability of the TRsandflies for the precise identification.

Results

The language of the program language was English for international use. In the program, there were 21 (14 quantitative and 7 qualitative) parameters for male sand flies and 17 (10 quantitative and 7 qualitative) parameters for female sand flies together with their maximum and minimum values (in mm and numerical) (Table 1). Not all the data were included in the database because some of them were not measured or ignored.

The web-based dynamic TRsandflies program consisted of 35 text boxes, 15 for male and 20 for female (Fig. 4) specimens, 130 photographs containing newly captured or archived jpg files and camera lucida drawings, and 24 distribution maps. TRsandflies's algorithm is developed in the Visual Studio compiler in the C# (Version 6.0) programming language, using the MYSQL database (Version 17.3) and Windows server. In addition, the program interface was coded in C# using HTML 5.0 and ASP.NET Framework 4.5.2 (Fig. 5). JavaScript and Bootstrap ready codes were used for the ease of use of the program. Communication between the user interface and MYSQL was provided with the C# usage.

TRsandflies had four pages: 1) Query Screen: The main screen that was queried according to the parameters in the database (Fig. 4); 2) Selection screen created for male and female specimens: Oriented screens depending on gender selection in the query screen (Fig. 4); 3) Result screen created for male individuals (Fig. 6); and 4) Result screen created for female individuals (Fig. 6). In addition, TRsanflies started to query by selecting one of the male and female options in the gender determination drop box on the main query screen. The number of parameters variations depending on gender selection and filtering was also provided.

Furthermore, the grid part of the query screen was filled with possible species by filtering process after the parameter textboxes were filled with the measurements of the sample to be determined by the user. In addition, the user was also provided with the ability of accessing the pages where the mouse hover feature was used, which includes microscope images, camera lucida drawings, distribution maps, vectorial capacity information, and systematic information to select the mentioned species from the grid. The user was also supported to make correct decision of species identification by comparing the information of the specimens with the information (Fig. 6) of the possible species.

Three validation of its performance on the identification of each sand flies species included in database was carried out for newly developed software. TRsandflies was also run 60 times for the male specimens, which were previously identified by conventional methods. It was observed that the number of species identified accurately was 52 (86.66%), whereas the number of false identification was 8 (13.33%). In addition, TRsandflies was run 60 times for female specimens similar to the trial of male specimens. The program identified the species correctly for 43 (71.66%) times, whereas it failed in the identification of species 17 (28.33%) times. On the other hand, it was not run for females and males of four *Transphlebotomus* species because of no healthy data.

TRsandflies was observed to be successful for the trial identification of the species, mostly in *Phlebotomus* subgenus specimens with 100% correct decision rate. It was also successful 100 and 93.33%, respectively, in the identification of the male specimens of the *Adlerius* and *Paraphlebotomus* subgenus.

Discussion

The application of web-based tools created for sand flies' identification is likely to be common in the future though they do not completely replace conventional methods. These new emerging

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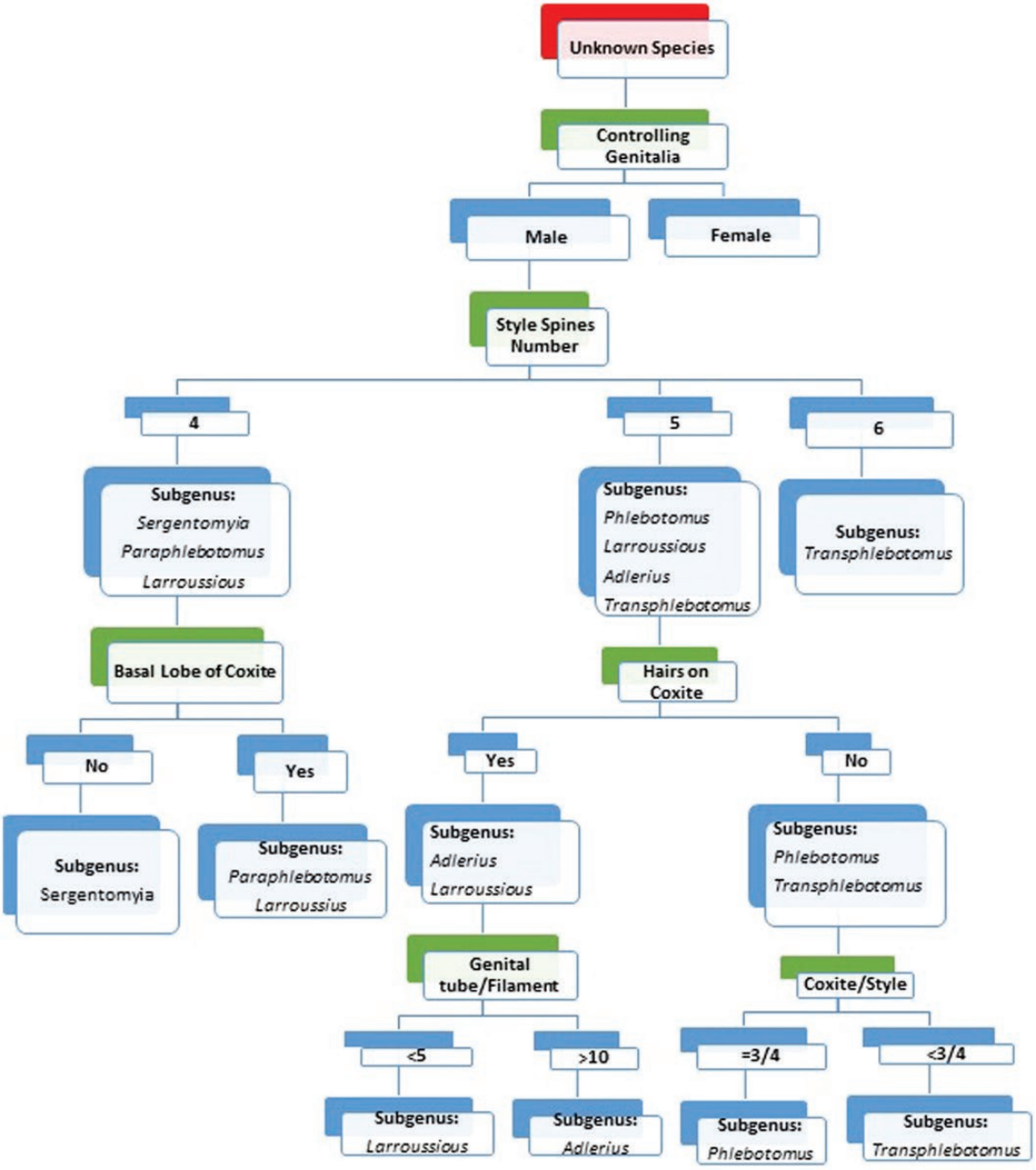


Fig. 2. TRsandflies 'algorithm for male sand flies.

technologies have an important role in the control of vector-borne diseases.

In the algorithm of TRsandflies which helped us summarize the morphological differences, different algorithms were produced according to sex differences. In the first steps of the TRsandflies algorithm, the morphological characters which was the most distinguishing feature between male and female specimens were used. The most important feature in distinguishing females in sand fly identification is whether spermatheca shows a segmented structure. Furthermore, in males, the number of spines in styles is the most important character in identification (Perfil'ev 1968).

This study was conducted in the light of the findings of previous field studies and compared my results with those of the field studies previously performed by other researchers. I have first reviewed the findings and found that the database of 24 species of sand flies identified before in Turkey. In this study, I only added the distribution data to the TRsandflies database due to the lack of sufficient data for the most recently identified species of *Transphlebotomus* subgenus (*Phlebotomus mascittii* Grassi, 1908, *Phlebotomus killicki* Dvorak, Votypka & Volf, 2015, *Phlebotomus anatolicus* Erisoz Kasap, Depaquit & Alten, 2015, and *Phlebotomus economidesi* Léger, Depaquit & Ferté, 2001) in Turkey (Kasap et al. 2015).

In a similar program developed by other researchers in Turkey, users were allowed to find unknown species by responding to the questions based on the anatomical features of sand flies. The most important difference between the TRsandflies and the

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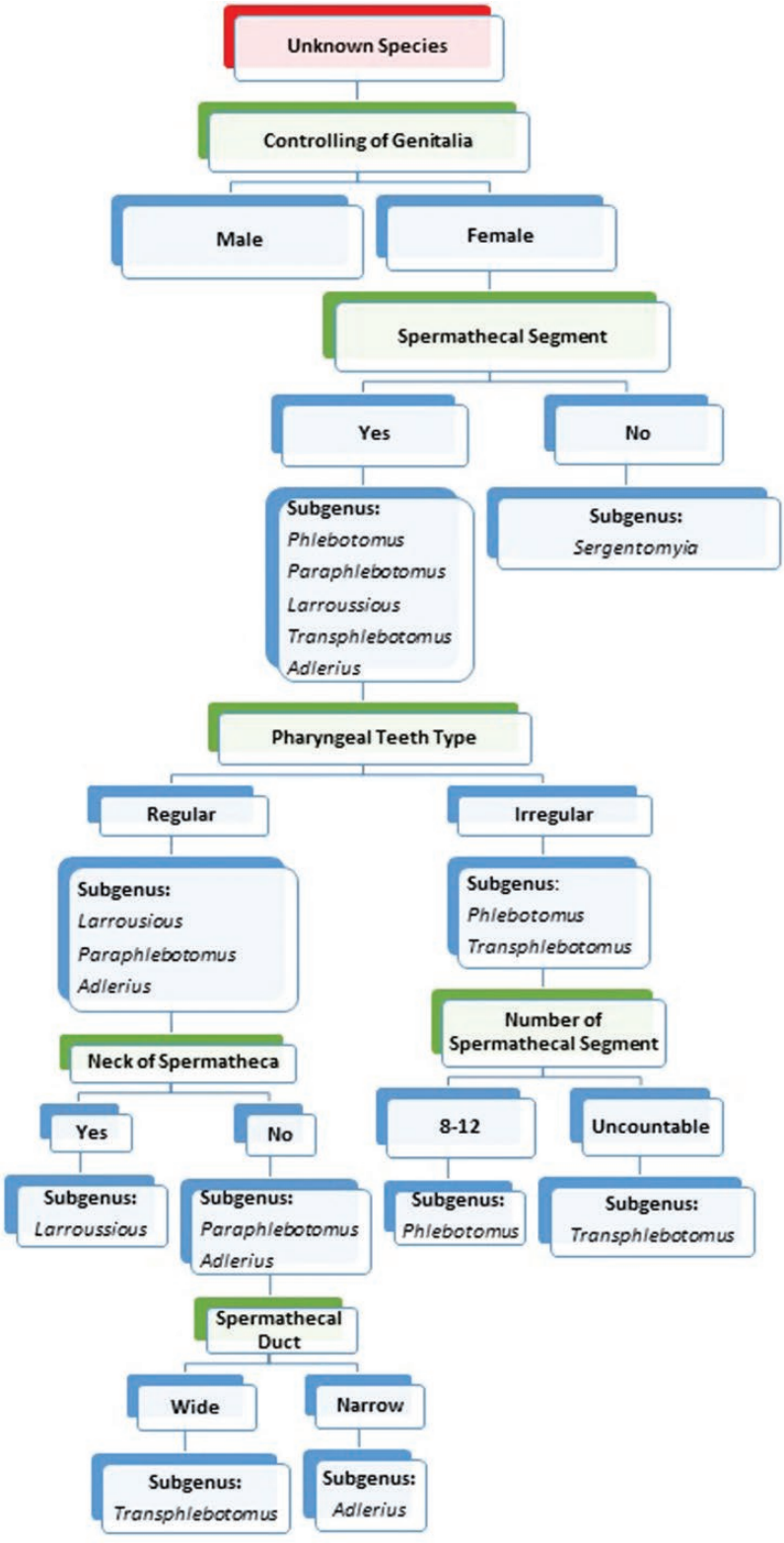


Fig. 3. TRsandflies 'algorithm for female sand flies.

above-mentioned one was that it was developed as a program where users could compare their measurements of sand flies and predict which species their measurements might belong to. In addition, more photos in the database of TRsandflies, and especially information (transmission, reservoirs, diseases, etc.) about the vectorial capacity of female sand flies, were among the important differences between

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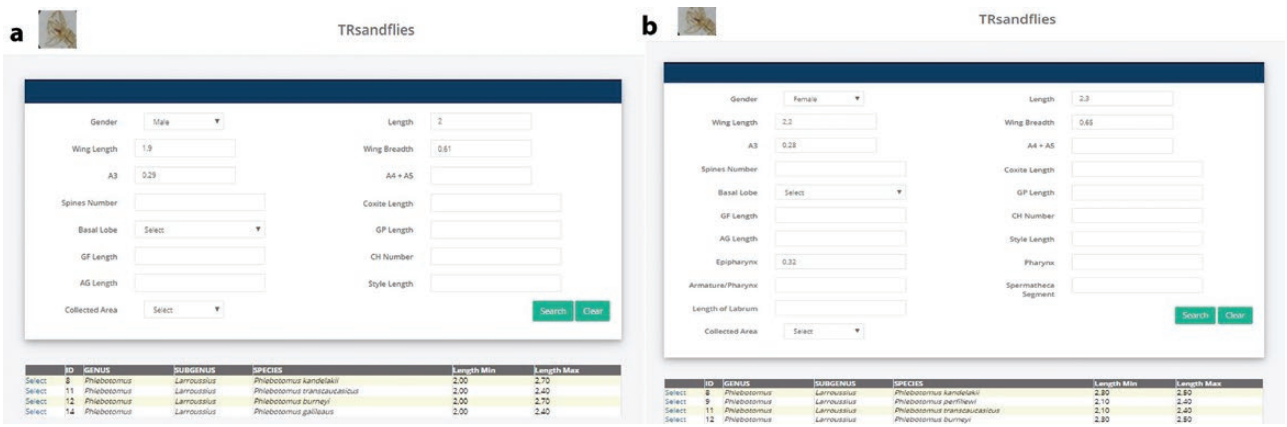


Fig. 4. The appearance of dropboxes and textboxes of sand flies in TRsandflies: (a) for male specimens and (b) for female specimens.

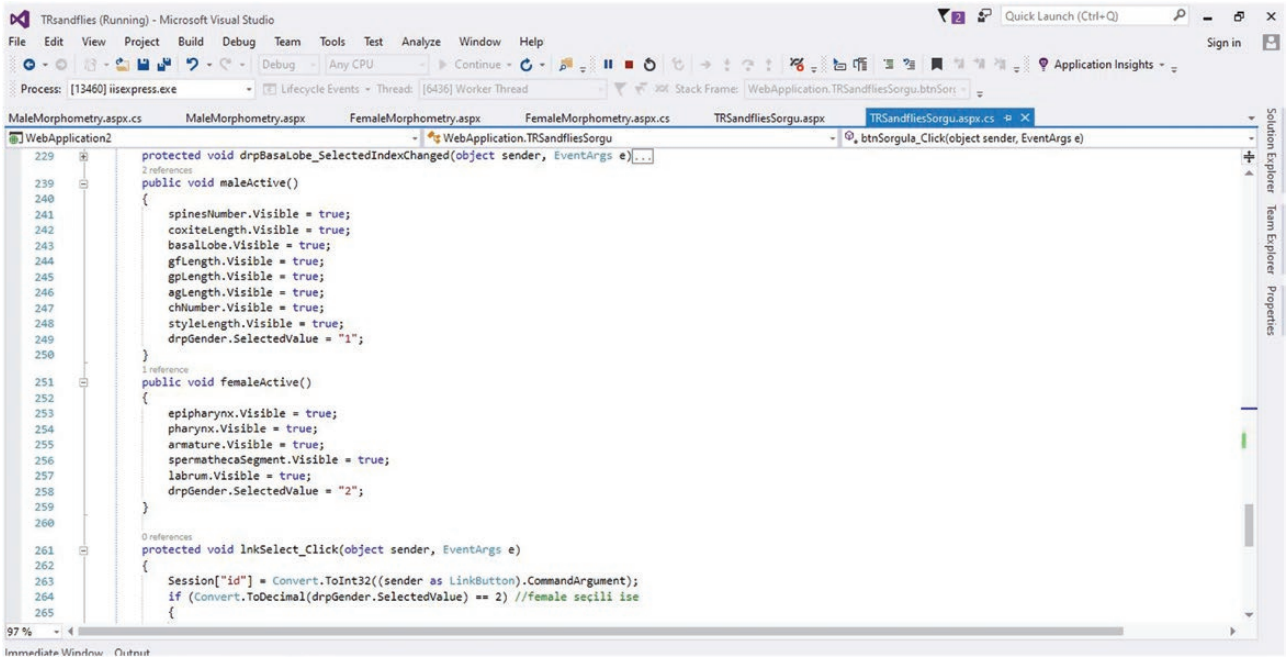


Fig. 5. TR sandflies' software in Visual Studio compiler using C# language.

the two programs. TRsandflies were written in the C# language and used the Windows server which made it different from others (Karakülah et al. 2016).

'Phlebotomes', a French-language interface which provided web-based identification of sand flies for the first time in the world, has systematic information, drawings, and pictures of 299 species recorded in Afrotropical regions. However, it provides information only about three of the species (*Phlebotomus papatasi* Scopoli, 1786, *Phlebotomus sergenti* Parrot, 1917, and *Phlebotomus alexandri* Sinton, 1928) identified in Turkey, though the program has reported more than one-fourth of the species of sand flies in the World (Niang et al. 2004).

In Turkey, there seems to be a conflict between the taxonomic status and distribution of the morphologically similar members of the *Ph. major* (*Ph. major* , *Ph. neglectus* and *Ph. syriacus*) complex. It is suggested that using a susbstantial number of morphometric characters or conducting molecular analyses using cytochrome *b* and elongation factor genes could be effective in marking the differences between these sister species (Kasap et al. 2013). In addition, I think TRsandflies program will only contribute to the researchers who make morphological identification, especially the researchers who study the *Ph. major* complex though molecular analyses have higher accuracy in sand fly identification than morphological analyses.

When tested by three students, TRsandflies was observed to have estimated the correct species for 52 times in 60 trials in male specimens and 43 times in 60 trials in female specimens in prediction of sand fly species. Unlike the SUS analysis which calculated the satisfaction rate and its contributions to the researchers of the first sand fly identification program developed in Turkey, the ability of TRsandflies was evaluated to find out how accurately it predicts species (Karakülah et al. 2016).

In conclusion, a web-based program, TRsandflies, whose predictive power was observed to be particularly high in male sand fly

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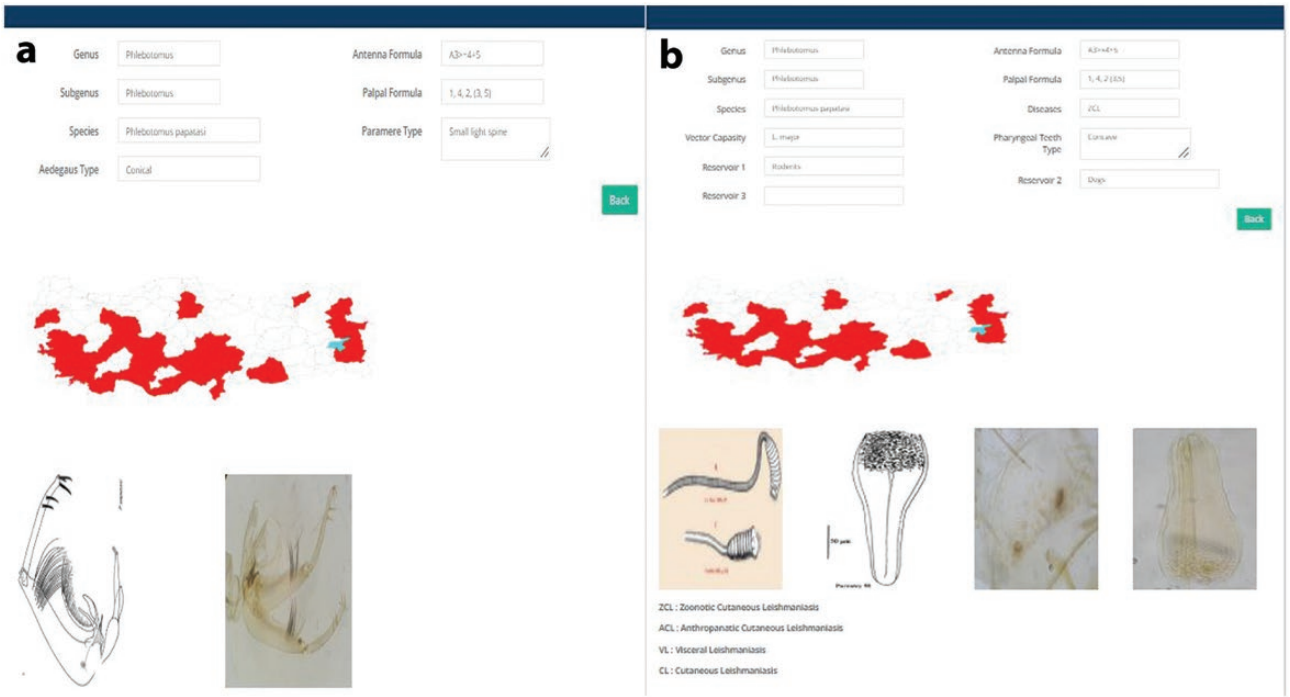


Fig. 6. The result screen: (a) male specimens of *Phlebotomus papatasi* result screen and (b) female specimens of *Ph. papatasi*.

specimens, has become one of the identification programs developed in Turkey. It is thought that the software which allowing the researchers to compare the measurement results with the database would provide users with satisfactory results in the diagnosis of leishmaniasis vector sand flies. In the forthcoming days, the program is planned to be delivered to users for free of charge by registering a domain.

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