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Research Article

Disseminated sandflies Sergentomyia and Phlebotomus in Al Muthanna province, Comparative and Taxonomic Study

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Abstract:

The taxonomy of the subfamily Phlebotominae has undergone several changes since the early twentieth century, and even with several studies using both old and new tools, including molecular markers, doubts remain about the relationship and differences between them. These differences reflect a lack of knowledge of the true evolutionary history of flies. In parallel, the old and current classifications consider only adult morphological data. However, there is consensus among the authors on the importance of morphological data from all life stages. These discrepancies illustrate the need for new studies to provide hypotheses that could contribute to the classification of this subfamily. Phlebotomies (Diptera, Psychodidae, and Phlebotominae) have been extensively studied, mainly due to the role of these flies as primary vectors of various parasites, including various *Leishmania* species, *Bartonella* and viruses that cause many diseases to humans and other vertebrates. The studies started to collect and identify the Phlebotominae sand fly present in different areas of Al-Muthanna province. The studies also aimed to determine their numbers and classify them based on the approved scientific classifications, as well as the difference between the most important species in the province. Light traps and sticky traps were used, and using the insect classification by (J. Abul-Hab and S. A. Ahmed, 1984) and (Al-Mayali & Al-Hassani,2016), and 4 species were recorded during a ten-month period. Within the identified four species, a total of 1486 specimens comprising four species of two genera were collected: *Phlebotomus* (two species) and *Sergentomyia* (two species). *Phlebotomus papatasi* 683 (45.967%) was the dominant species followed by *Sergentomyia sentoni* 380 (25.57%), *Phlebotomus sergenti* 290 (19.51%). *Sergentomyia squamipluris* 133 (8.95%) distributed among 822 females and 664 males.

Keywords: *Phlebotomus*, *Sergentomyia*, sandflies, Taxonomy

Introduction

Sand flies are insects belonging to the order Diptera, subfamily Phlebotominae, which present hematophagous feeding habits. There are about (900) species already described, and (70) of these have been reported as potential vectors of Leishmania spp. With a few others involved in the natural transmission of viruses, such as Phlebovirus belong to (Reoviridae) family (Ayhan, Prudhomme, Laroche, Bañuls, & Charrel, 2020). These

insects are still able to harbor other microorganisms, because they are in contact with different environments and substrates. This aspect can be particularly important because many of these microorganisms that make up the insect microbiota can also carry viruses (Ferreira & Aguiar, 2018)**..** Different species of sand fly live in diverse environments. The order Diptera, or true flies, constitute 12-15% of animal species, and are the most ecologically diverse of the various insect species (Badii, 2020). Sand flies are classified in oder :Diptera, suborder Nematocera, family Psychodidae and subfamily Phlebotominae (Maneeroth, Noonanant, Thongkhao, & Pengsakul, 2020). The identification of sand fly species is epidemiologically very important because less than 10 percent of over 800 species described are responsible for the transmission of human pathogens such as flagellate protozoa of the genus *Leishmania* (Flaih, 2022). Blood-sucking arthropods transmit a variety of human pathogens acting as disseminators of the so-called vector-borne diseases. Leishmaniasis is a spectrum of diseases caused by different *Leishmania* species, transmitted quasi worldwide by sand flies (Cecílio, Cordeiro-da-Silva, & Oliveira, 2022).

Being the only established vectors of the protozoan parasites of the genus Leishmania, sand flies have become very important in all countries where leishmaniasis exists. It is caused by a variety of species, each one having specific mammalian reservoir hosts and vectors. Leishmania and sand fly classification has always been a controversial matter, and the increasing number of sand fly species described world complicates the task. The English denomination refers to the pale (sandy) colour of this insect. There is further confusion because in certain parts of the world, midges of the genus Culicoides (Diptera: Ceratopogonidae) and black flies (Diptera: Simuliidae) are referred to by the same name. A distinction must therefore be made for the vectors of the leishmaniases and other diseases of public health concern, which are correctly termed ‘phlebotomine sandflies’(Lafri & Bitam, 2021). . More importantly, the phlebotom sandfly is the biological vector for most of the Leishmania parasites that still cause many disfiguring pests and claim thousands of lives each year in more than 90 endemic countries (Adly et al., 2021). .

Sand flies are classified in oder Diptera, suborder Nematocera, family Psychodidae and subfamily Phlebotominae (Benallal, Garni, Harrat, Volf, & Dvorak, 2022). The dominance of these insects as vectors is differential between vertebrates; thus, they have a leading role in transmitting viruses, protozoans, and other pathogens to mammals and birds; however, they have a more discrete representation as vectors of parasites among ectotherms (Sasidharan & Saudagar, 2021) .As sand flies have medical importance, their correct taxonomic identification is a crucial component of any epidemiological study (Chavy et al., 2019). Presently, the identification and classification of phlebotomine sand fly species depends on comparison of external and internal morphological characters. However, this approach has frequently led to the misidentification of individuals within sibling species or among species that present very closely related morphology (Șulești et al., 2021). The taxonomy of the group has a great epidemiological importance since not all species are proven vectors, and the ones that have been proven so far possess differences in bite behavior or host preference. Therefore, the clarification of the taxonomic and systematic classification of the group is the main purpose of mostly all studies involving sandflies (Vojtková, 2022).

The classification of sand flies is still a matter of some controversy and there is no universally accepted system (Munstermann, 2019) . A World Health Organization expert group concluded that taxonomy of sandflies is an essential basis for all work on sandfly biology and role as vectors of *Leishmania* parasites (Șulești et al., 2021), and classified into six genera of *Phlebotomus, Sergentomyia, Chinius, Warelyia, Lutzomyia,* and *Brumptomyia* (Saghafipour et al., 2017). Phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) are hematophagous insects. Some species are vectors of several viral, bacterial, and protozoal diseases, especially leishmaniasis. In the last few decades, new proposals for classification and identification of Phlebotominae sand flies have been based primarily on adult morphology. Three genera of sand flies, Phlebotomus, Sergentomyia and Lutzomyia are very important from medical point of view. The former two genera are present in Old World (Afro-Eurasia) while the latter is the principal genus in New World (North and South America) (Galati, 2018).

The geographical distribution of sand fly species is the key factor determining the geographical distribution of these diseases. Although sand flies are active during the warm months, they spread their activity geographically and for longer periods of time during the year due to favorable climatic changes. This allows them, and the pathogens they carry, to reach new hosts and regions. Sand flies are small, delicate insects and use sheltered places to rest during the day, being active mostly at dusk and night (Alten et al., 2016) . They have Avery strong adaptation capacity and a plastic reaction norm against changing environmental and climatic conditions. Even though they can fly relatively short distances from their breeding sites, some species have been reported to travel up to 2.3 km. Sand flies are mostly found in rural and peri-urban environments and not at high altitudes since they require warm temperatures (Tsirigotakis et al., 2018).

This study aimed to apply specific taxonomic methods to identify the types of sand flies and differentiate some species based on some taxonomic characteristics of insects that were caught in specific areas in the city of Al-Muthanna.

Materials and Methods

Sand flies samples were collected from the regions by geographical location of places that expect the existence or appearance of sand flies. The major consideration in the selection of the study sites is that they represent different ecological regions with different environmental conditions that may have an effect on the distribution and the population genetics of the sandflies species. A survey of phlebotomine sand fly populations was carried out in several areas of Almuthanna provinies . The sites of survey were samawa, Alrumaitha, Alwarka, Al-majed, Al-Khidir, Al-hilal, Alnajme . four light trap baited were set up at each location from 7:00 PM to 7: 00 AM every seven days during 2021 and 2022. Sticky traps constitute a method of sampling sand flies by interception rather than attraction (Figure 1). These traps are generally inexpensive and easy to manufacture in large numbers. Sticky traps were preferred over light traps to collect sandflies from indoors since they are non-attractive and are only interceptive traps.

These traps were paper labels A4(210 × 297 mm) coated on both sides with diesel and castor oil and fixed vertically on sticks held at 120 cm above the ground level. The traps

were fixed during the evening in one hour before the sunset and collected by the next morning. Sandflies captured by these traps on the papers were removed using small brushes, washed in dilute detergent and preserved individually in 70% alcohol in Eppendorf tubes with proper labeling until processing and identification in the laboratory later, and only these were selected for studying their morphological shape

Figure 1. Sticky traps

Species identification

The sandflies were processed in the conventional way. The sandflies were thoroughly washed twice with distilled water to remove castor oil and excess hairs from the sandflies. Sand fly specimens were preserved in 1.5 ml Eppendorf tubes with 70% ethanol with drops of glycerin to prevent stiffening of insects, and an attached label that recorded the site and day of sampling.

External morphological analyses of the habitus of different species were carried out by the mounted sand fly on every slide and drops of mounting media DPX medium were added and the cover slip was gently put on the specimen. These slides of sand flies were prepared for morphological identification using standard keys, The description of taxonomic structures for most common sand fly species, as well as those that spread in Iraq according to (Al-Mayali & Al-Hassani, 2016) as following:

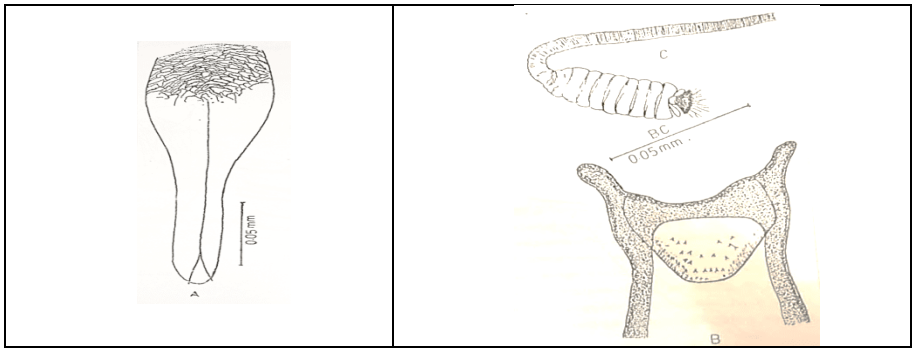

Figure 2. *Phlebotomus papatasi* Female.A- pharynx , B- Cibarium , C- Spermatheca

Sandflies has about 2000 species, divided into three genera *Phlebotomus*, *Sergentomyia* and *Lutzomyia*; the second genus *Sergentomyia* has no medical importance because it depends on feeding on animals; the third genus, *Lutzomyia*, is spread only in Americas (New World), while the first genus *Phlebutomus* attacks humans and other animals and

transmits many pathogens. In Iraq, about 17 species of sand fly were identified. in Iraq, morphological studies showed that *P. papatasi* is a predominant sand fly species and hypothesised to transmit CL causing Leishmania species including *L. major* and *L. tropica*. Few studies have found Leishmania species in sand flies in mixed pools of samples in this country(Al-Bajalan, Niranji, Al-Jaf, & Kato, 2021) . The species of the vector sand fly is still not very well solved. (Al-Awadi, 2019) suggested that both *P. papatasi* and *P. alexandrimight* be the vector.

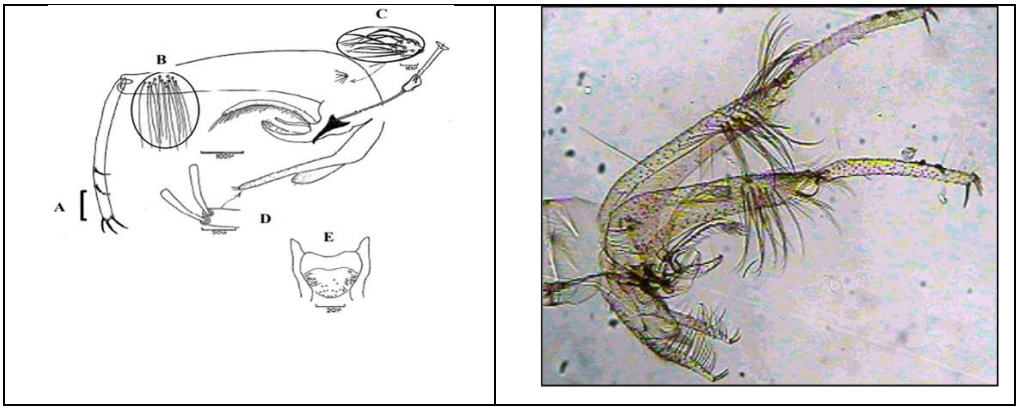

Figure 3. Phlebotomus papatasi Male. (A) Spines position. (B) Coxite setae. (C) Tuft of seta. (D) Setae of the lateral lobe. (E) Cibarium. F- Terminal of male(Ježek, Tkoč, Oboňa, Manko, & van Harten, 2021).

If the posterior end of the abdomen looks sharp like this, then it is a female. We noticed that the females do not have any external appendages on the edge of the abdomen. So, when you see any supplements, it is almost certainly male. Males have a large appendage (reticulated structures) at the end of their abdomen. Not all male peripherals are as obvious as the examples above, you will often need to look closely as they are often small and closed like a pocket knife, especially in newly emerged males.

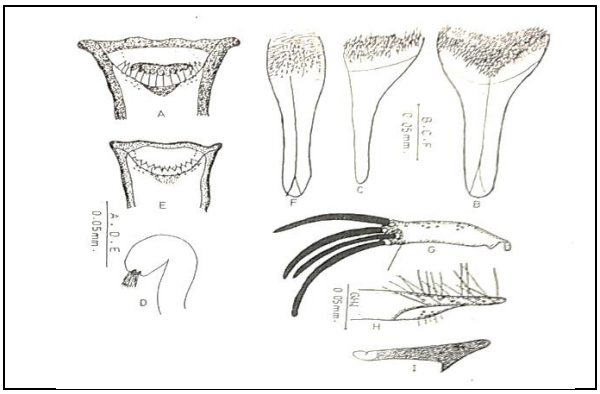

Figure 4. Sergentomyia *sitoni.* (Female), A- Cibarium, B- pharynx, C- Alaterd ventral plate of pharynx, D- Spermatheca. (Male), E- Cibarium , F- pharynx, G-Style, H-Paramere , I- Aedeagus.

The morphological characters of the sand flies head such as antennal flagellumers and its associated structure have been used for differentiating of Phlebotominae sand flies at inter and intra-species levels.Traditional morphometric investigations and multivariate analysis of morphometric characters have been used to resolve taxonomic problems in Phlebotominae sand flies, especially in identifying the closely related species such as isomorphic or cryptic species (Godoy et al., 2018).

Results and Discussion

Four species were recorded during a period of ten months using the classification of insects (Al-Mayali and Al-Hasani, 2016) and according to the local and international standard classifications. A total of 1486 samples comprising four species of two sexes were collected (Fig. 5.a). Bloodletting (two types) and Sergentomyia (two types). *Phlebotomus Papatasi* 683 (45.967%) was the dominant type followed by *Sergentomyia sentoni* 380 (25.57%), *Phlebotomus sergenti* 290 (19.51%). *Sergentomyia squamipluri* 133 (8.95%) distributed among 822 females and 664 males (Fig. 5.b).

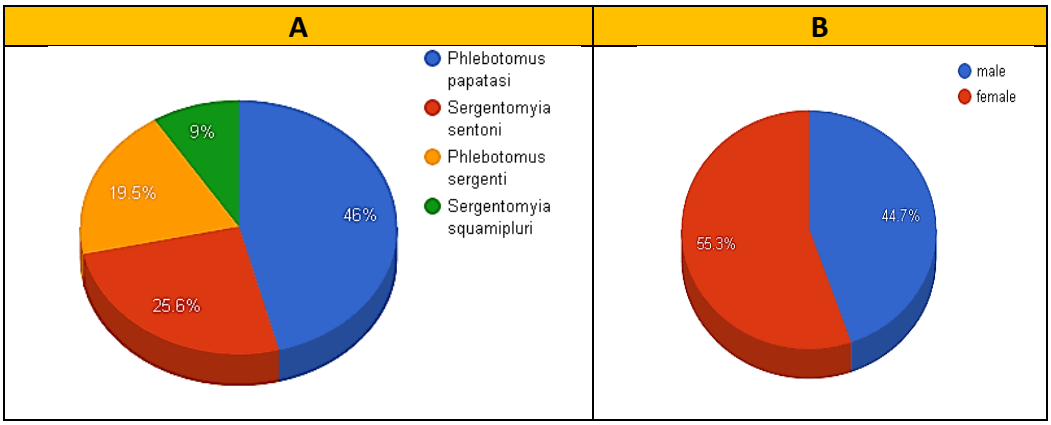

Figure 5. (A)Distribution of species in Al-muthanna province. (B) Distribution of species according gender

The following is a description of the taxonomic structures of the four most common sandfly species obtained in the province. One type of Phlebothomus (Phlebotomus papatasi) and another type of *Sergentomyia* (*Sergentomyia sintoni*)were selected for the purpose of classifying and comparing the genera.

Phlebotomus papatasi

The female of this species has been diagnosed by spermatheca, a cylindrical capsule, divided into several sections, they are about 8-12 sections wide, and those near the duct are slightly smaller, the seminal duct is striped. Figure 6. Sperm are shown with 8-12 rings (short apical section), Fig. 7. It shows that the pharyngeal motor does not extend beyond the posterior third, with scaly teeth arranged in a wide interlocking network.

Figure 6. *Phlebotomus papatasi* Female. A- Spermatheca

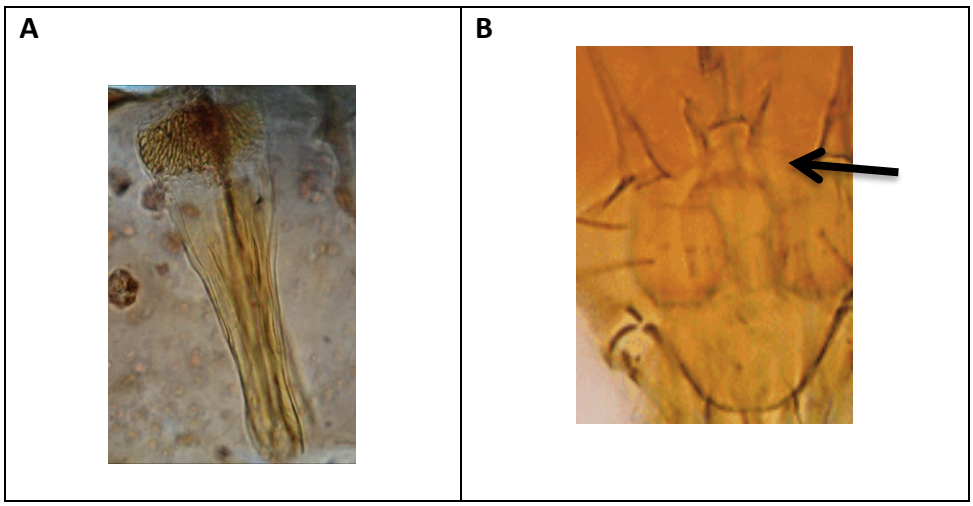

Figure 7. *Phlebotomus papatasi* Female.A- pharynx, B- Cibarium

Males were diagnosed by the end of the genitals, pharynx, and basal hairs found in Superior Claspers, as well as the pattern with five spines. Style with five short spines: 3 apical spines and 2 external spines in the apical third. Paramir with 2 dorsal ramifications. Surstyle with 2 short distal spines (Figure 8.c). Male genitalia contain genital filament, genital pump, and Aedeagus (Figure 8.B). Aedeagus short, Conical, apex curved, Lateral lobe with two short terminal spines. The paramere contain ventral, dorsal and median lobs. The dorsal lob longer the ventral, and the median shorter than ventral lob (Figure 8.A).

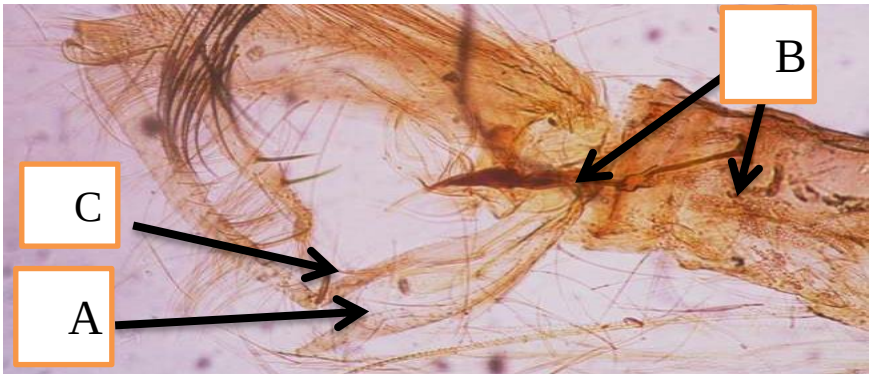

Figure 8. Phlebotomus papatasi Male. A- paramere, B- Genitalia, genital Filament, genital pump, and aedeagus, C-style with basal spines and terminal spines.

Our results are consistent with several studies indicating that the prevalence of Leishmaniasis largely depends on the distribution of invertebrate vectors, and identification of sandfly species prevalent in endemic and surrounding areas is important and essential for predicting disease risk. Sandflies are generally identified as adults based on morphological features, particularly internal structures such as spermatogonia, stoma, and pharynx in females, and terminal genitalia in males. Therefore, elucidation of group classification is often the main goal of all studies involving sand flies. Some morphological structures, which are taxonomically important for both males and females, are adopted.

Sergentomyia sintoni

The experiment was also carried out on females diagnosed by Spermatheca, not segmented, tubular, smooth, broad, and apex with shallow eversion, cibarium with 12-16 weak teeth of the same size. Dark brown pigment patch, apex at some point with narrow pale anterior protuberance, small notch mid-base. Nasopharyngeal funnel like (Figure 9.).

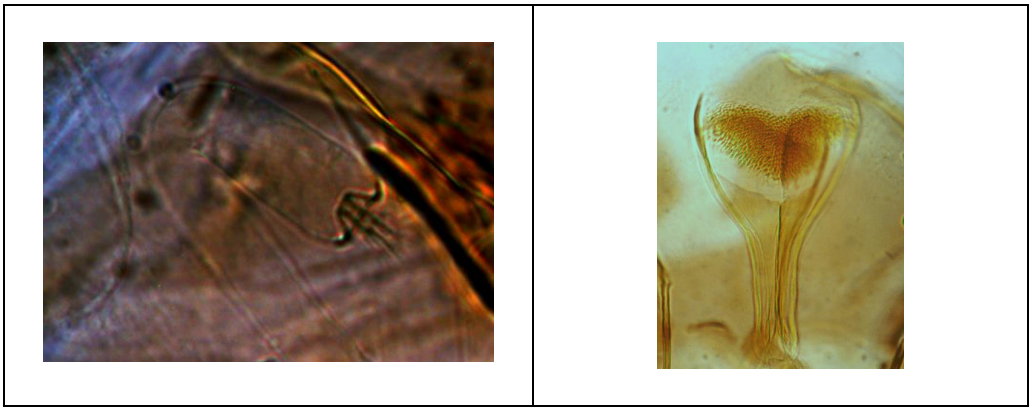

Figure 9. Sergentomyia *sentoni* Female, A- Spermathecae 40x, B- pharynx 40x

The males were diagnosed through the Style contains four terminals spines (Figure 10). two terminals and other quasi-terminal. The internal terminals are one larger than the other, and the longer the style itself, and other spines about us long as style, and other Three spines along the length of the style. male genitalia contain Two parts are external and internal. The external are genital pump, and internal Ejaculatory duct. paramere long, pointing hand-like. Aedeagus long, apex papilla like. cibarium has one or more rows of teeth and the pigment patch is usually present.

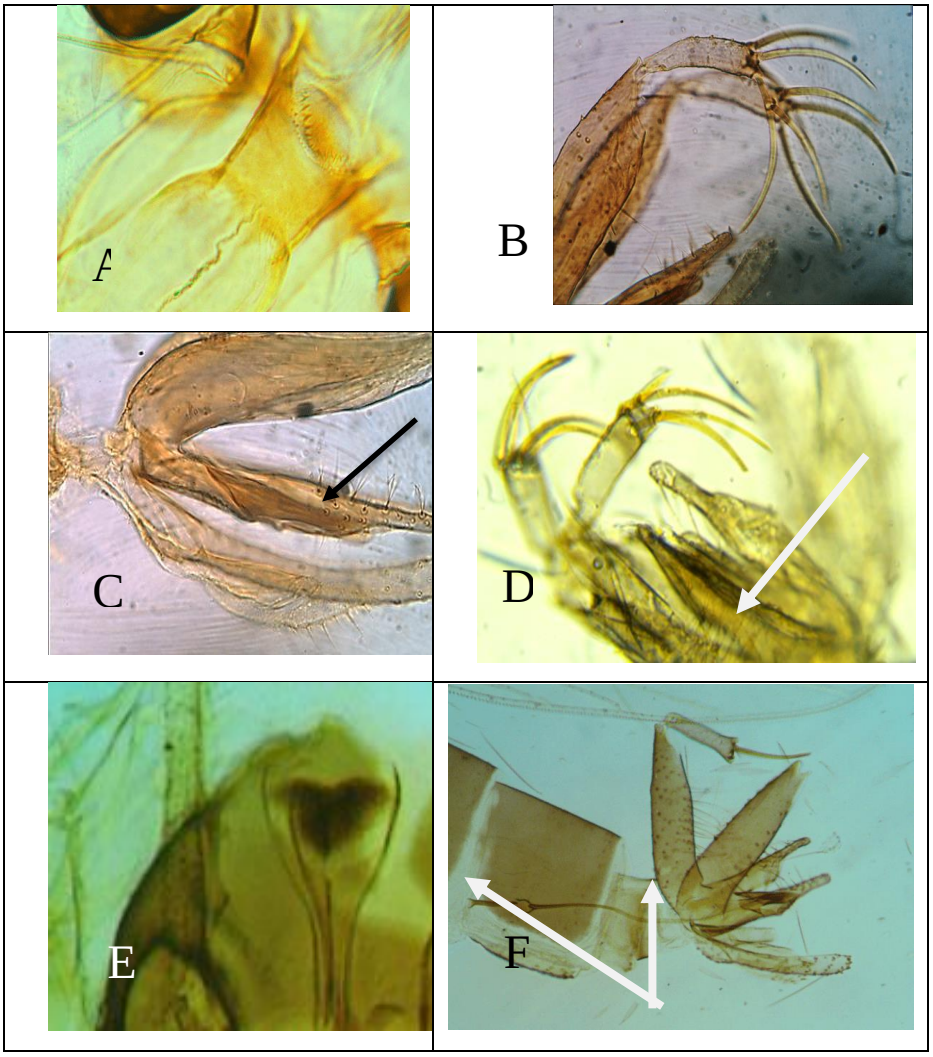

Figure 10. Sergentomyia *sentoni* Male. A- cibarium 40x, B- Style 40x , C- paramere 10x D- Aedeagus 10x, E- pharynx 40x, F- Male genitalia 40x.

Sergentomyia mervynae was first reported from Karbala Town, Iraq by Pringle (1953). Pringle while describing female specimens did not supply morphometric measurements of cibarium and pharynx and figures of mouthparts, antennal segments, palps and wings. Similarly, morphometric measurements of cibarium and pharynx and illustrations of antennal segments, wings, palps, coxite, surstyle, genital filament and pump of male fly were also not supplied (Pringle, 1957). Due to the lack of a large-scale phylogenetic study on the taxonomy of the Old World sand flies, there is no synapomorphy of the *Sergentomyia* genus. Consequently, it is difficult to define this genus with exactitude; it is more a convention than a scientific group. The presence of cibarial teeth in the females of this genus is one of the most used characters to include species in the *Sergentomyia* (Buatong et al., 2022) .

This species was found inside and outside the houses .little numbers in outside , and this is consistent with previous field research found by (Al-Mayali & Alyasiri, 2020) . These results were similar to what found in Isfahan and Kashan city in Iran (Zahraei-Ramazani & Leshan, 2016). As well as ,found the same results in Kaleybar, East-Azerbaijan province, Iran (Firouzjaie et al., 2023) .Also, these are the first keys prepared for Sri Lankan sand fly fauna, and include 20 species of sand flies from two genera

(Phlebotomus and Sergentomyia) reported in the country features (Wijerathna & Gunathilaka, 2020). The genus *Sergentomyia* is mainly distributed in the Old World tropics (Nawaz et al., 2020). The distribution beginning with May in a few numbers, and then began to multiply in July. This is consistent with a study conducted in Diwaniya Iraq (Al-Mayali & Al-Hassani, 2016). *Sergentomyia sentoni* is located in rural areas. Which are abundant with stockyard, It is close to what was reported by a study of several Iranian cities , who explained *S. sintoni* might predominate in villages and in the burrows & holes for the local reservoir host of L. major that called *gerbil Rhombomys opimus* (Al-Ardi, 2022).

Conclusion

In conclusion, the present research provides identification keys for genera and species of phlebotomine sand flies found in Al muthanna province, which will facilitate future studies on these medically important insects. These simplified keys, along with the line drawings provided herein are intended for anyone dealing with sand fly identification.

Conflict of Interests.

The authors declared that no competing interests exist .

Ethical Issues.

In this research, ethical considerations have been fully observed.

Acknowledgement.

The success and end result of this task requires a lot of guidance and help from many people, and we are very fortunate to have had this throughout the course of the work. All we have done is only due to these directions and help. This research could not have been completed without the participation and assistance of so many people whose names we may all be missing. Their contributions are sincerely appreciated and gratefully acknowledged

Authors' Contributions.

The research idea was written and designed by the above-mentioned researcher and submitted to the journal.

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