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Morphological and Morphometric Taxonomic Identification of Phlebotomine Sand Flies (Psychodidae) in Selected Areas of the Al Jabal Al Gharbi Region, Libya

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التعرّف التصنيفي المورفولوجي والمورفومتري لذباب الرمل الفليبوتومي (*Psychodidae*) في مناطق مختارة من إقليم الجبل الغربي، ليبيا

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Abstract
Leishmaniases represent a significant public health problem due to their serious socioeconomic and psychosocial impacts in endemic areas. This study aimed to conduct a comprehensive taxonomic assessment of sand fly species in northwestern Libya based on morphological and morphometric analyses. A total of 353 sand fly specimens were collected during 2025. Species identification and classification were carried out using morphological and morphometric characteristics. The results revealed the presence of ten sand fly species belonging to two genera, including six species of Phlebotomus and four species of Sergentomyia.
Morphometric measurements of the recorded sand fly species were compared across three geographical localities (Kalipa, Al-Rabta, and Al-Qawasim) to evaluate spatial variation. Eight morphometric characters were measured and analyzed, allowing the identification of reliable diagnostic traits that effectively discriminated among the ten sand fly species recorded in northwestern Libya.

Keywords: Leishmaniasis – Sand flies – Morphometrics – Leishmania – Vector identification – Libya..

الملخص

تُعدّ داء اللشمانيات مشكلةً مهمة في مجال الصحة العامة نظرًا لما تُحدثه من آثار اجتماعية واقتصادية ونفسية خطيرة في المناطق المتوطنة. هدفت هذه الدراسة إلى إجراء تقييم تصنيفي شامل لأنواع ذباب الرمل في شمال غرب ليبيا اعتمادًا على التحليلين المورفولوجي والمورفومتري.

تم جمع ما مجموعه 353 عيّنة من ذباب الرمل خلال عام 2025، حيث جرى تحديد الأنواع وتصنيفها باستخدام الخصائص الشكلية والقياسات المورفومترية. وأظهرت النتائج وجود عشرة أنواع من ذباب الرمل تنتمي إلى جنسين هما: ستة أنواع من جنس Phlebotomus وأربعة أنواع من جنس Sergentomyia.

كما تمت مقارنة القياسات المورفومترية للأنواع المسجّلة في ثلاث مناطق جغرافية (كليبة، الربطة، القواسم) بهدف تقييم التباين المكاني. وقد جرى قياس وتحليل ثماني صفات مورفومترية، مما أتاح تحديد صفات تشخيصية موثوقة مكّنت من التمييز الدقيق بين الأنواع العشرة من ذباب الرمل المسجّلة في شمال غرب ليبيا.

الكلمات المفتاحية: داء اللشمانيات – ذباب الرمل – القياسات المورفومترية – Leishmania – التعرف على النواقل – ليبيا.

Introduction

Phlebotomine sand flies (Psychodidae) are medically important insects and represent the primary vectors of *Leishmania* parasites, the causative agents of leishmaniasis, a globally distributed zoonotic disease affecting both humans and animals. Transmission occurs when an infected female sand fly takes a blood meal from a vertebrate host, inoculating the promastigote stage of the parasite, which subsequently transforms into the amastigote stage within host macrophages. This process results in a wide spectrum of clinical manifestations, ranging from self-healing cutaneous lesions to severe and potentially fatal visceral infections (Cecílio et al., 2022; WHO, 2023).

Accurate identification of sand fly species is fundamental to understanding the epidemiology of leishmaniasis, as vector competence varies markedly among species and specific *Leishmania* parasites are transmitted by particular sand fly vectors. In addition, environmental and climatic factors, including temperature, humidity, and habitat characteristics, strongly influence the geographical distribution and seasonal dynamics of sand fly populations, thereby shaping local transmission patterns (Ready, 2013; Galati, 2018; Tesfaye et al., 2025).

Morphological and morphometric studies provide effective and widely accepted tools for the identification of sand fly species in both males and females. These approaches allow for the discrimination of diagnostic characters among closely related species and play a critical role in taxonomic, ecological, and epidemiological investigations. Traditional morphological-based methods have proven essential for assessing sand fly biodiversity and for understanding spatial variation in species distribution across different ecological settings (Lewis, 1982; Lane, 1993; Killick-Kendrick, 1999; Galati, 2018).

In Libya, particularly in the northwestern region, information on sand fly species diversity and geographical distribution remains limited, despite the area being endemic for cutaneous leishmaniasis. This lack of comprehensive taxonomic data constrains accurate assessment of vector species involved in disease transmission. Therefore, detailed taxonomic studies based on morphological and morphometric analyses are essential to establish a reliable scientific framework that supports epidemiological surveillance and the development of effective vector control strategies aimed at reducing leishmaniasis transmission in this endemic region.

Literature Review

Phlebotomine sandflies (Psychodidae) are medically significant insects, serving as the primary vectors of *Leishmania* parasites that cause leishmaniasis, a zoonotic disease affecting humans and animals (Cecílio et al., 2022). The subfamily Phlebotominae includes two Old World genera, *Phlebotomus* and *Sergentomyia*, and three New World genera, *Lutzomyia*, *Brumptomyia*, and *Warileya* (Young & Duncan, 1994). In the Old World, *Phlebotomus* species are the only medically relevant vectors, while *Sergentomyia* primarily feeds on reptiles (Lewis, 1982). Morphological traits such as the cibarium, antennal segments, palps, and wing venation are key for species differentiation (Triplehorn & Johnson, 2005).

In the Mediterranean basin, nine species of the subgenus *Larroussius* (*P. perniciosus*, *P. ariasi*, *P. perfiliewi*, *P. neglectus*, *P. langeroni*, *P. tobbi*, *P. longicuspis*, *P. kandelakii*, *P. syriacus*) are proven or probable vectors of canine leishmaniasis (Killick-Kendrick, 1990; Guilvard & Rioux, 1991). Their distribution varies according to ecological niches, which affects local transmission patterns. Laboratory colonies have been established for six of these species to study their biology and vector competence (Killick-Kendrick et al., 1991).

In Libya, at least 21 sandfly species have been recorded, with *P. papatasi* and *P. longicuspis* implicated as vectors of zoonotic cutaneous leishmaniasis (ZCL) in the northwestern region (El-Buni et al., 1993; Ashford et al., 1976; Obenauer et al., 2012). Recent surveys in Al Rabta villages reported nine sandfly species (six *Phlebotomus*, three *Sergentomyia*), with *P. papatasi* and *P. sergenti* being the most abundant and active from June to October, coinciding with peak CL cases (Dokhan et al., 2016).

Cutaneous leishmaniasis caused by *L. major* and *L. tropica* has been endemic in northwestern Libya for decades, with outbreaks recorded periodically, including over 3,500 cases between 1990–1992 and over 7,000 cases in 2006 (Aoun & Bouratbine, 2014). Studies have confirmed the presence of *L. tropica* in districts such as Nalut, Misrata, Jabal Al Gharbi, and Tarhouna (Amro et al., 2012; Belal et al., 2012). These findings highlight the importance of accurate species identification and monitoring to guide effective vector control strategies.

Aims of the Study

Although leishmaniasis has been studied in various regions, comprehensive research on its vector, phlebotomine sandflies, remains limited, particularly regarding morphological classification and morphometric variation. This study aims to:

- Identify and classify sand fly species in selected areas of the Al Jabal Al Gharbi region using morphological traits and morphometric measurements.
- Evaluate the effectiveness of morphometric measurements as a reliable tool for distinguishing sand fly species across different sites.

Materials and Methods

Study Area

The study was conducted in selected areas of the Al Jabal Al Gharbi region, northwestern Libya. This region is characterized by a mountainous terrain forming part of the Nafusa Mountain range and is located approximately 80 km south of Tripoli. The study sites included three localities: Al-Rabta, Kalipa, and Al-Qawasim, which were selected to represent different ecological settings within the Al Jabal Al Gharbi region.

The area lies at an approximate altitude of 300 m above sea level and is geographically located around 32°9′N and 12°50′E. Sand fly sampling was carried out over an extended period from

April 2024 to October 2025 to ensure adequate spatial and temporal coverage for morphological and morphometric analysis.

Sandfly Collection

Sandflies were collected outdoors at night using CDC light traps (Model 512, John W. Hock, Gainesville, FL, USA). Traps were placed approximately 30 cm above the ground before sunset and retrieved the following morning. Samples were transported to the laboratory for morphological analysis.

Sandfly Processing and Morphological Identification

Collected sandflies were preserved in 70% ethanol, cleaned in chloral hydrate:phenol (1:1, vol/vol), and mounted in Puri's medium. Specimens were examined under a dissecting microscope, sorted by sex, and identified to species level using standard identification keys (Lewis, 1982; Lane, 1986; Annajar, 1999; Kakarsulemankhel, 2010). Morphological characters were measured using a calibrated ocular micrometer under an Olympus compound microscope (10X and 40X objectives), and measurements were converted to millimeters (Singh et al., 2007).

Results

Species Composition

A total of **353 sandflies** were collected and identified in this study, representing **ten species** belonging to two genera: *Phlebotomus* (6 species) and *Sergentomyia* (4 species). The identified species are as follows:

Genus: Phlebotomus

- **Subgenus Phlebotomus:** *Phlebotomus papatasi* (Scopoli)
- **Subgenus Paraphlebotomus:** *Phlebotomus sergenti* (Parrot), *Phlebotomus alexandri* (Sinton)
- **Subgenus Larroussius:** *Phlebotomus longicuspis* (Nitzulescu), *Phlebotomus langeroni* (Nitzulescu), *Phlebotomus* sp. (Larroussius group)

Genus: Sergentomyia

- **Subgenus Sergentomyia:** *Sergentomyia minuta* (Rondani), *Sergentomyia fallax* (Parrot), *Sergentomyia antennata* (Newstead)
- **Subgenus Sintonius:** *Sergentomyia clydei* (Sinton)

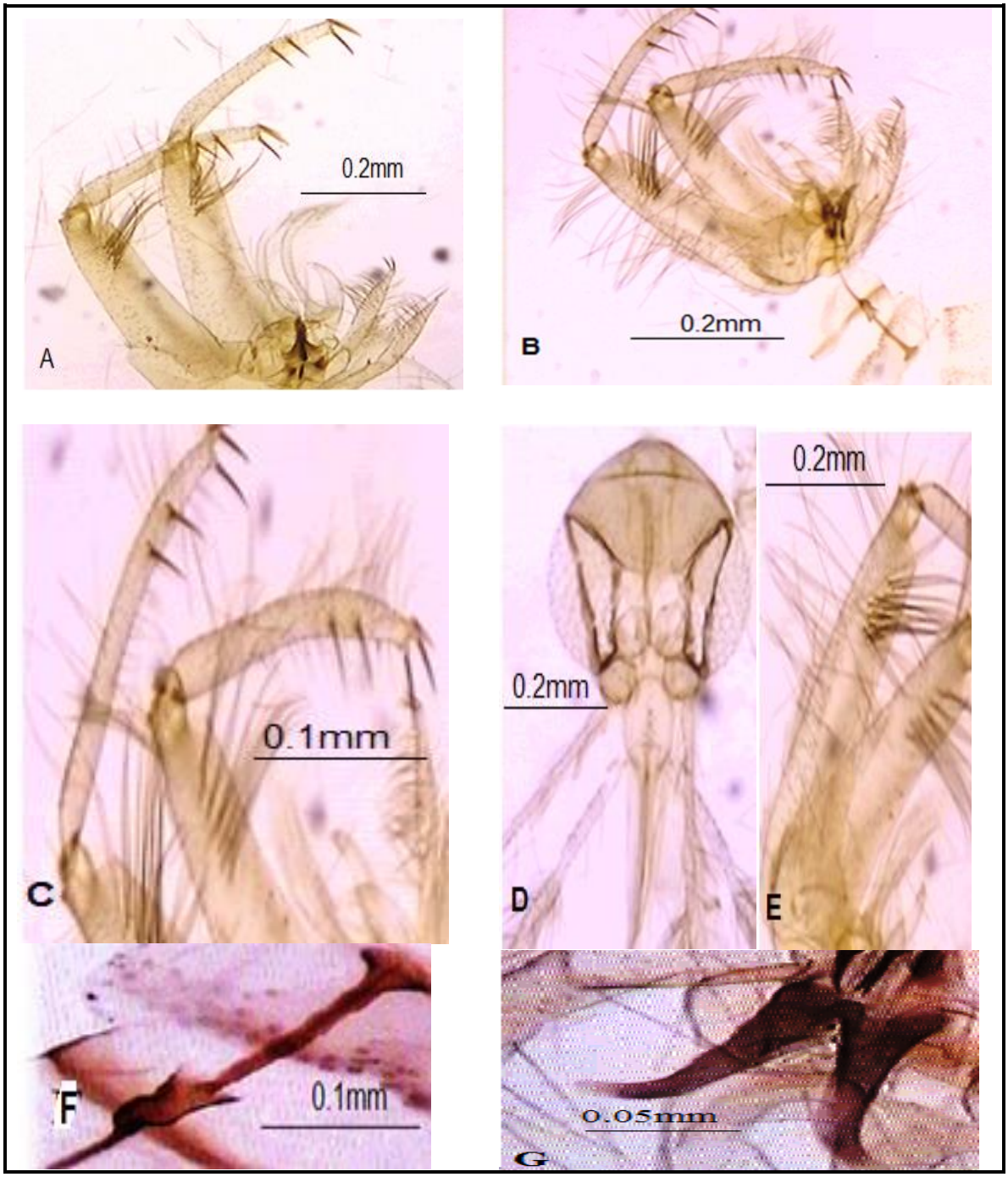


Figure 1: *P.papatasi,* Male: A & B, whole genitalia C, style, D, Whole head, E, coxite F, genital pump; G, aedeagus; [Specimens collected from Kalipa and Alqawsim and AI Rabta area of the NW region. ofLibya].

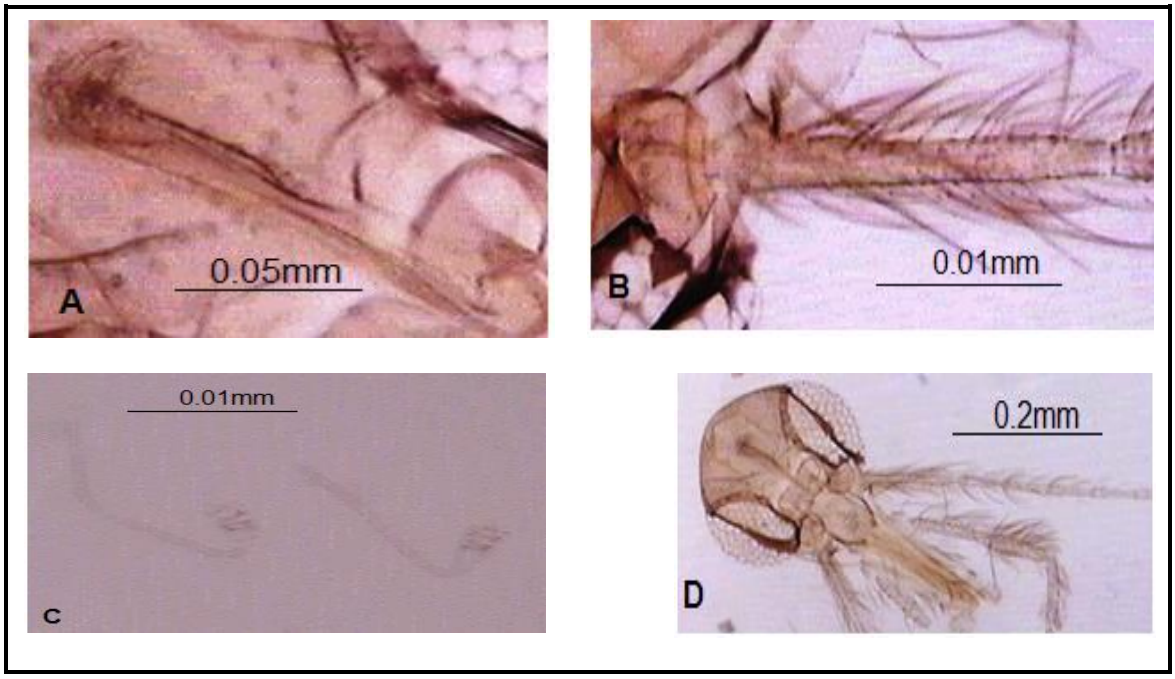


Figure 2: *P.papatasi,* Female:A, pharynx;B, antenna segment, C, spematheca; D, Whole head; [Specimens collected from Kalipa and Alqawsim and AI Rabta area of the NW region of Libya].

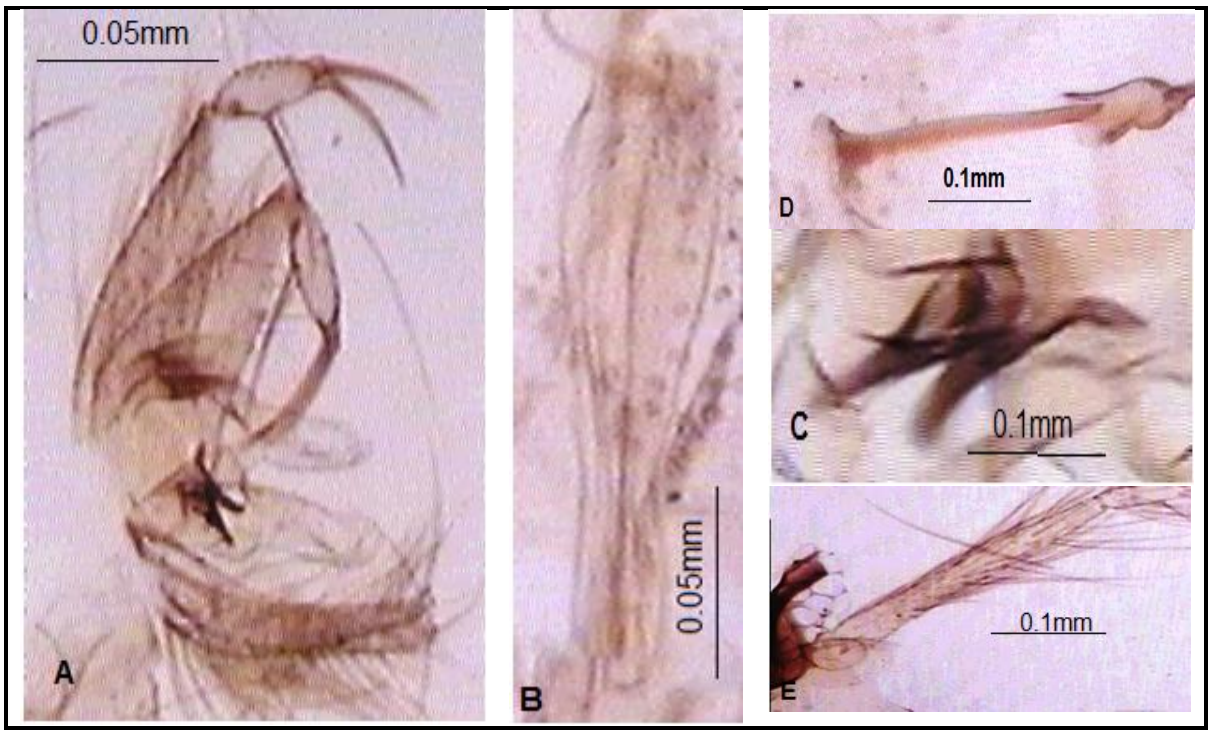


Figure 3: *P. sergenti*, Male: A, whole genitalia; C,aedeagus; D,genital pump, Female: B, pharynx, E;antenna segment. [Specimens collected from AI Rabta and Kalipa and Alqawsim area of the NW region of Libya].

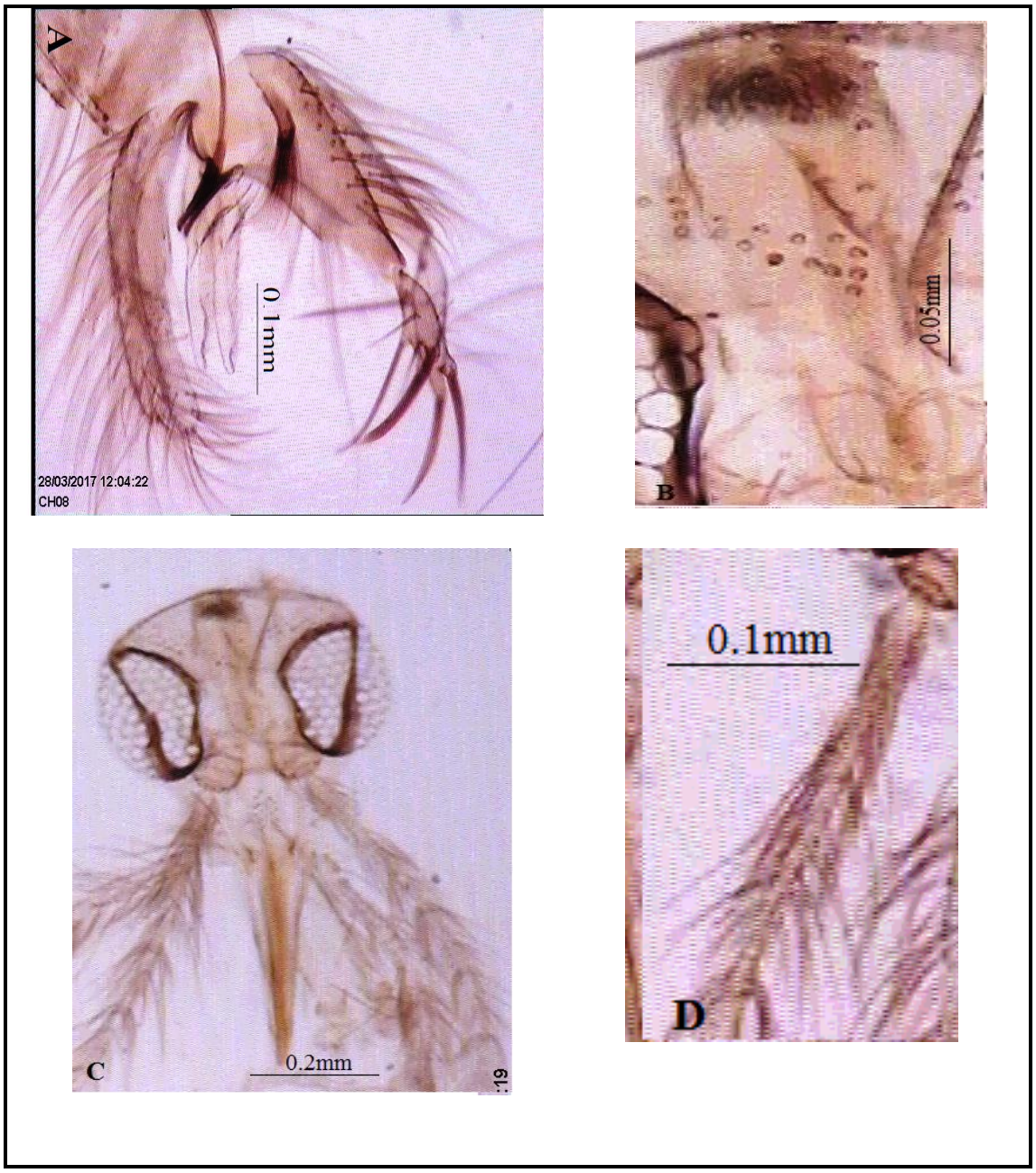


Figure 4: *P.alexandri*,Male: A, wholegenitalia;D;antenna segment Female:B,pharynx C, whole head. [Specimens collected from AI Rabta and Kalipa and Alqawsim area of the NW region of Libya].

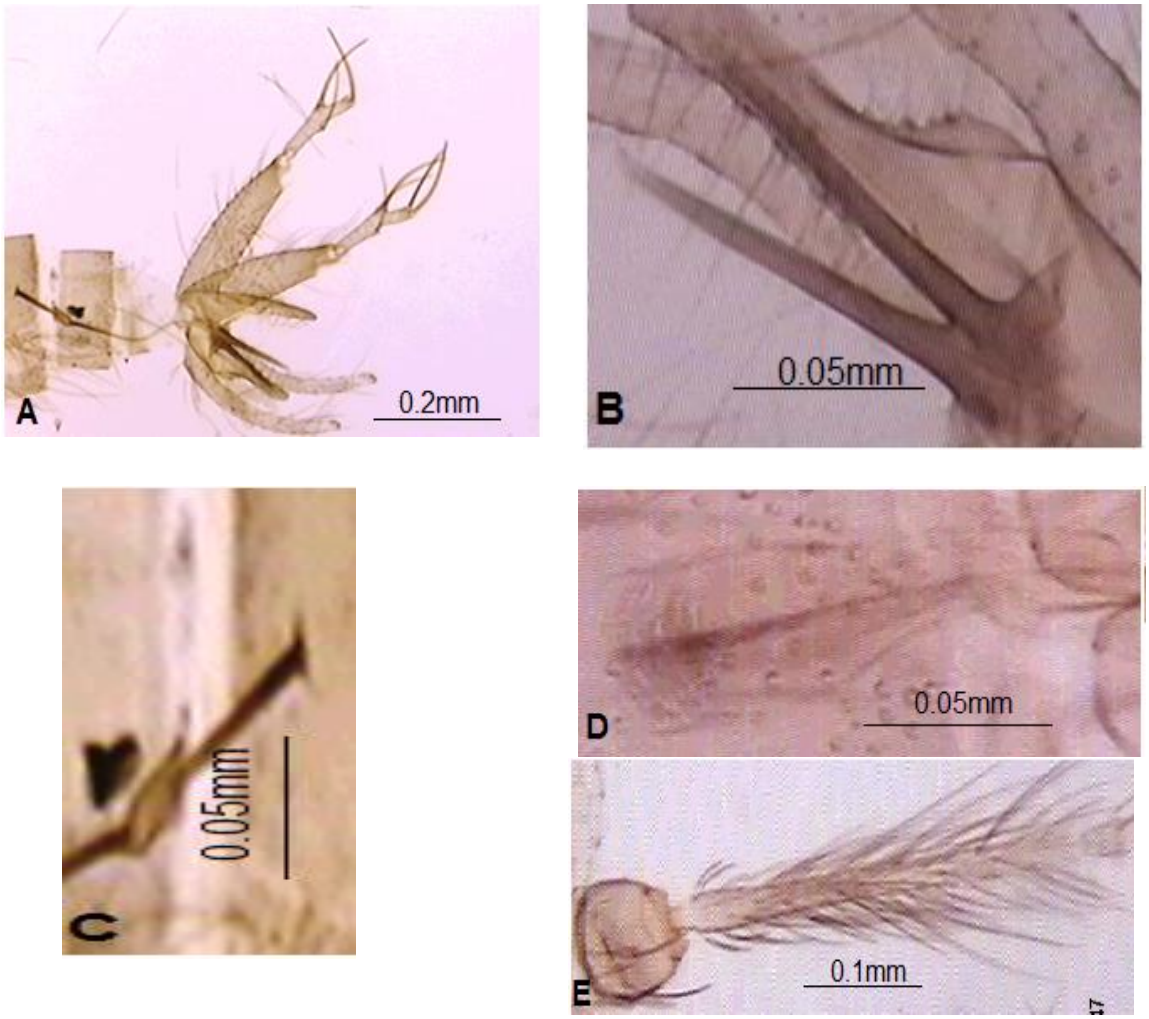


Figure 5: *P. langeroni,* Male: A,whole genitalia; B, aedeagus; C,genital pump; female: D, pharynx; E; antenna segment. [Specimens collected from AI Rabta and Kalipa and Alqawsim area of the NW region of Libya].

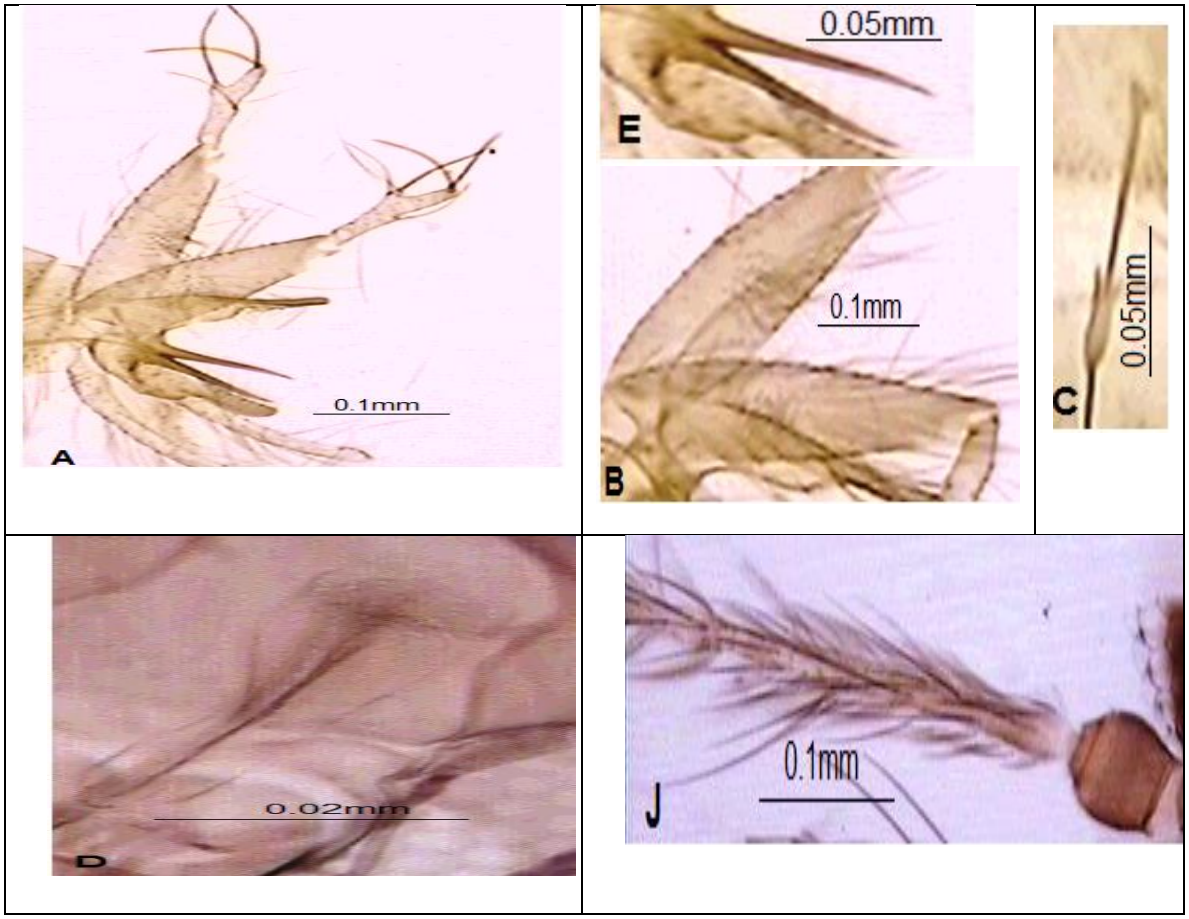


Figure 6: *P.longicuspis*,Male: A, whole genitalia; B. coxite. C,genital pump E,aedeagus F, paramere. Famale: D, pharynx; J. antenna segment [Specimens collected from AI Rabta and AIqawsim area in the NW region of Libya].

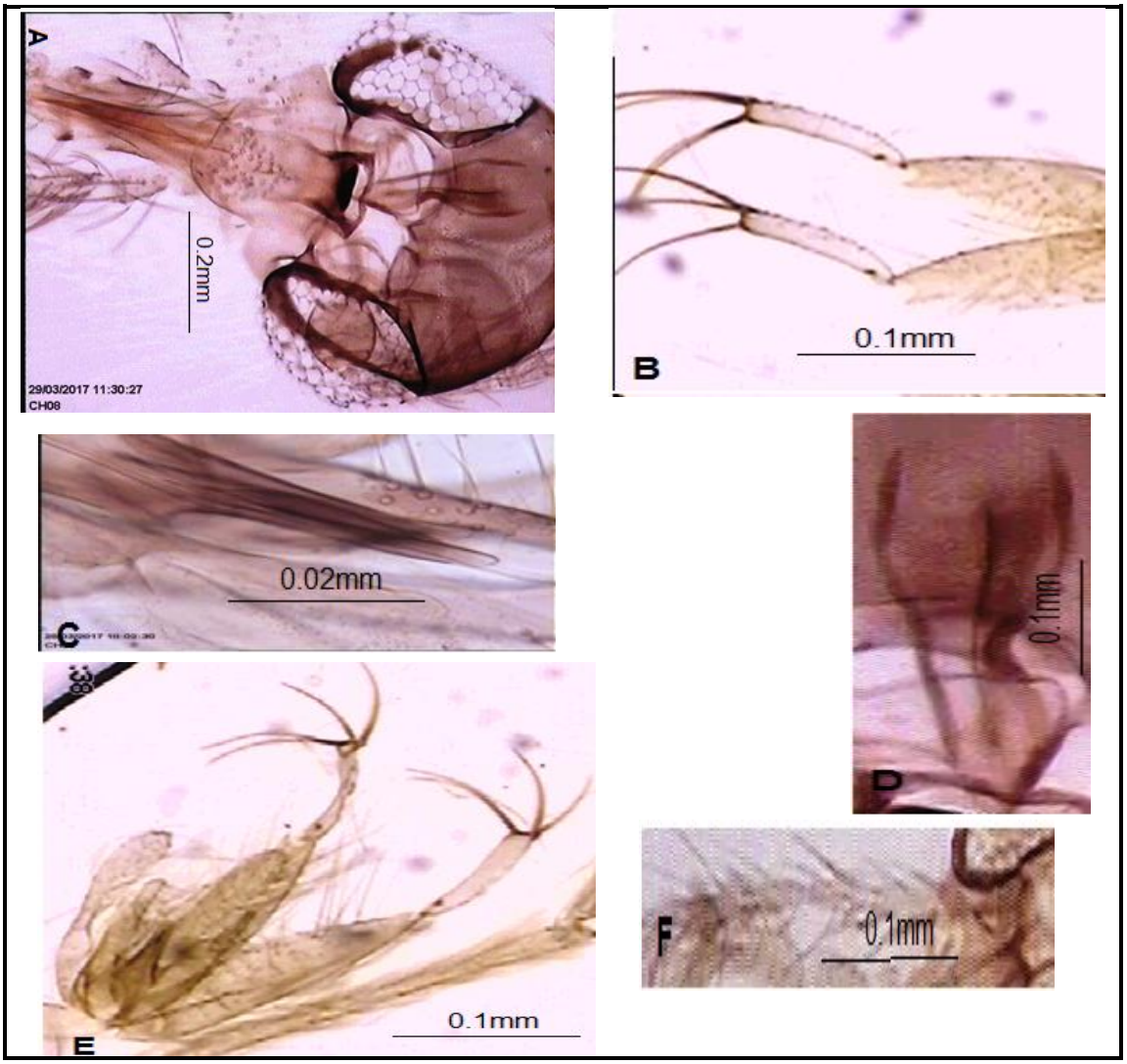


Figure 7: *S.minuta,* Male: B,style; C, aedeagus .E, whole genitalia,. Female: A,whole head; D,pharynx, F,antenna segment [Specimens collected from Kalipa and Alqawsim AI Rabta area in the NW region of Libya].

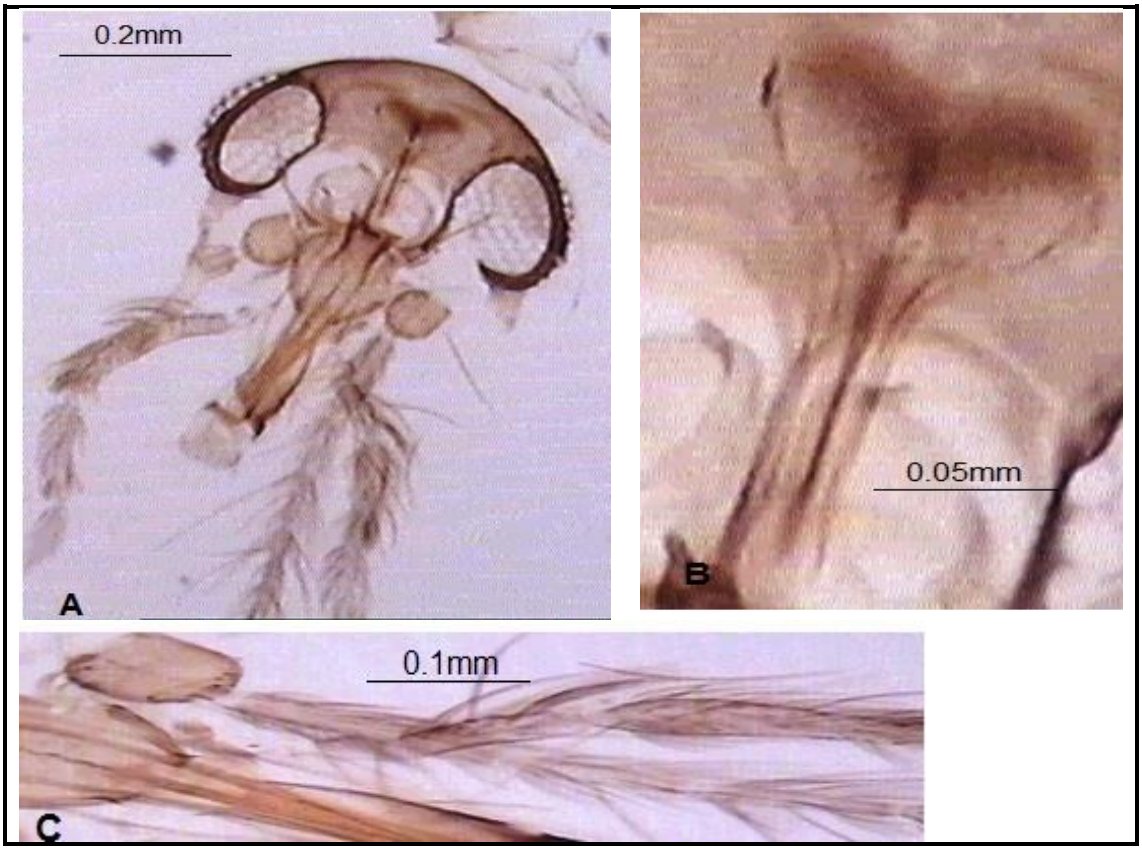
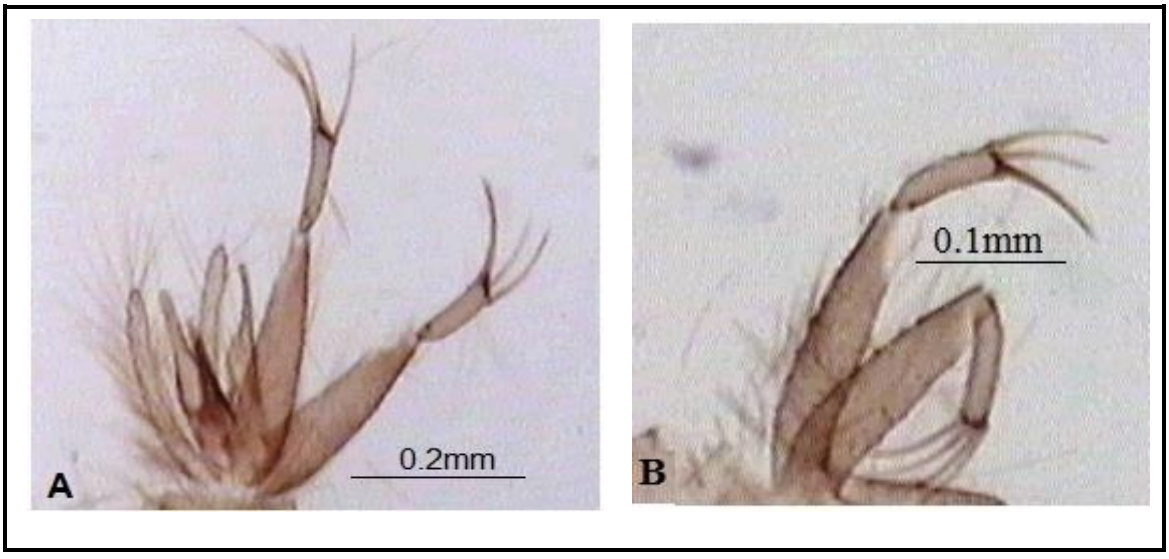


Figure 8: *S. fallax*,Female: A,whole head; B,pharynx;C, antenna segment [Specimens collected from AI Rabta area in the NW region of Libya].



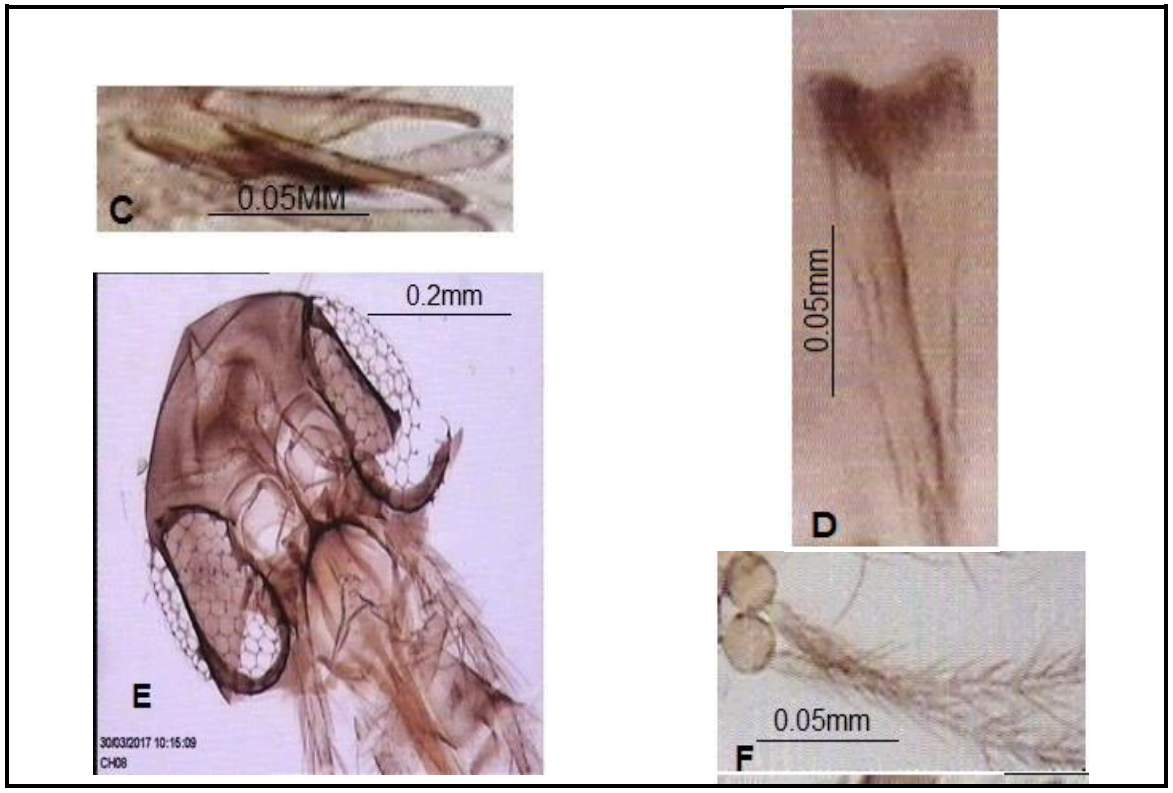
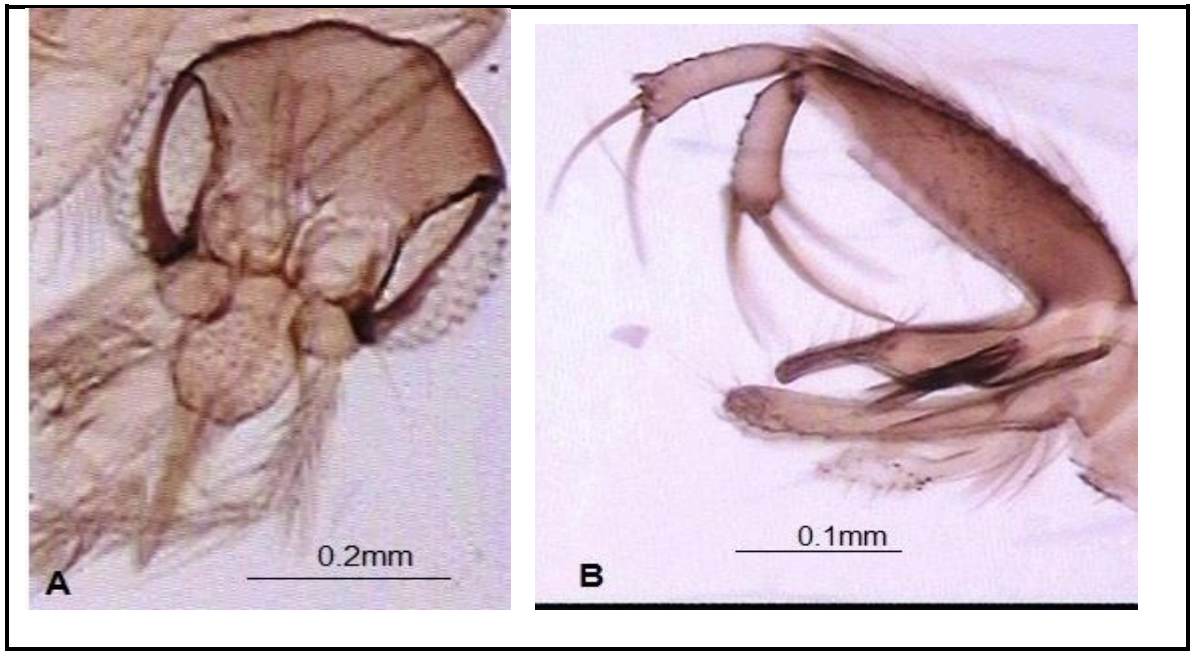


Figure9: *S.antennata*,Male :A, whole genitalia, B,style; C,aedeagus.Female:D,pharyn pharynx; E,whole head,F, antenna segment. [Specimens collected from Kalipa and Alqawsim AI Rabta area in the NW region of Libya].



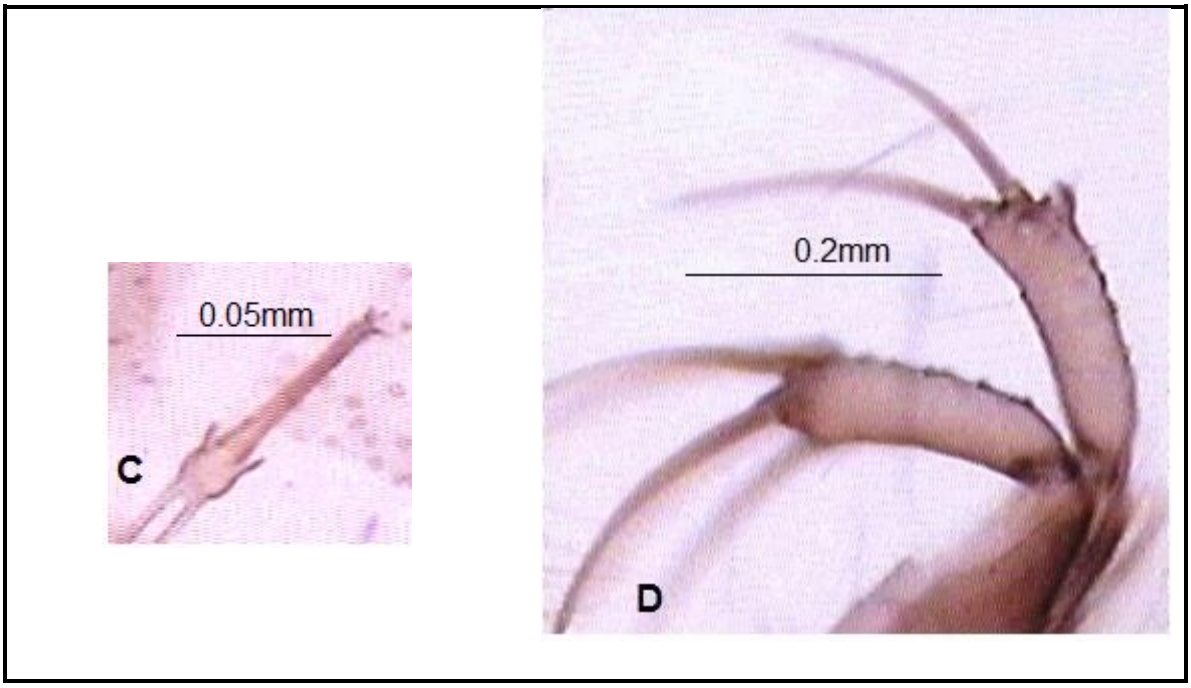


Figure10 : *S.clydei*, Male:A,whole head; B,Whol genitalia; .C, genital pump.D, style. [Specimens collected from Kalipa and AI Rabta area in the NW region of Libya].

1. **Morphometric Characters of Sand Fly Species**
2. **Morphometric Differentiation Among Sand Fly Species**
3. **Female Antennal Measurements**

The total lengths of female antennae across the different sand fly species collected from all study sites are presented in Table 1. The recorded mean values were as follows: *Phlebotomus papatasi* (0.28 mm), *P. sergenti* (0.26 mm), *P. langeroni* (0.27 mm), *P. longicuspis* (0.24 mm), *P. alexandri* (0.30 mm), *Sergentomyia minuta* (0.15 mm).

These values indicate the typical average antennal lengths for females of each species, reflecting a consistent morphometric pattern across all study areas.

Table 1. Measurements of female antenna.

Species	Samples per area			Total	Antenna length (mm)	
	Kalipa	ALRabta	Alqawasim		Mean±SD	Range
P. papatasi	13	7	10	30	0.22 ± 0.07	0.09-0.35
P. sergenti	8	13	9	30	0.25 ± 0.05	0.17-0.33
P. langeroni	5	25	0	30	0.27±0.03	0.22-0.31
P. longicuspis	0	30	0	30	0.26 ± 0.03	0.22-0.29
P. alexandri	11	7	0	18	0.30 ± 0.04	0.24-0.35
S. minuta	20	9	1	30	0.15 ± 0.02	0.13-0.17
F (5,162)				175	36.97, P < 0.0001	

Female Pharyngeal Measurements

The total lengths of the female pharynx for the different sand fly species collected from all study areas are presented in Table 2. The recorded mean values were as follows: *Phlebotomus*

papatasi (0.25 mm), *P. sergenti* (0.27 mm), *P. langeroni* (0.22 mm), *P. longicuspis* (0.22 mm), *P. alexandri* (0.28 mm), *Sergentomyia minuta* (0.18 mm), *S. fallax* (0.17 mm), and *S. antennata* (0.21 mm).

These measurements represent the typical average pharyngeal lengths for females of the examined species, indicating consistent morphometric characteristics across all study sites.

Table 2. Measurements of female pharynx.

Species	Samples per area			Total	Pharynx length (mm)	
	Kalipa	ALRabta	Alqawasim		Mean±SD	Range
P. papatasi	13	7	10	30	0.26 ± 0.03	0.20-0.31
P. sergenti	8	13	9	30	0.24 ± 0.03	0.20-0.29
P. langeroni	5	25	0	30	0.23 ± 0.02	0.21-0.26
P. longicuspis	0	30	0	30	0.23 ± 0.04	0.17-0.29
P. alexandri	11	7	0	18	0.27 ± 0.03	0.22-0.31
S. minuta	20	9	1	30	0.18±0.01	0.16-0.20
F (5,162)				175	34.18, P < 0.0001	

Male Antennal Measurements

The total lengths of the male antennae for the different sand fly species collected from all study areas are presented in **Table 3**. The recorded mean values were as follows: *Phlebotomus papatasi* (0.34 mm), *P. sergenti* (0.35 mm), *P. alexandri* (0.32 mm), *P. langeroni* (0.28 mm), *P. longicuspis* (0.28 mm), *Phlebotomus* sp. (0.32 mm), *Sergentomyia minuta* (0.16 mm), *S. antennata* (0.16 mm), and *S. clydei* (0.21 mm).

These measurements represent the typical average antennal lengths for males of the examined species, indicating consistent morphometric characteristics across all study sites.

Table 3. Measurements of male antenna.

Species	Samples per area			Total	Antenna length (mm)	
	Kalipa	ALRabta	Alqawasim		Mean±SD	Range
P. papatasi	15	0	15	30	0.27 ± 0.01	0.26-0.29
P. sergenti	15	0	15	30	0.32 ± 0.04	0.26-0.38
P. alexandri	10	0	0	10	0.34 ± 0.01	0.32-0.37
P. langeroni	15	9	6	30	0.25 ± 0.03	0.20-0.31
P. longicuspis	0	13	17	30	0.24 ± 0.03	0.17-0.32
S. minuta	12	8	10	30	0.17 ± 0.01	0.15-0.19
F ≈ 34				160	F(5,154) ≈ 34.0, P < 0.0001	

Male Pharyngeal Measurements

The total lengths of the male pharynx for the different sand fly species collected from all study areas are presented in Table 4. The recorded mean values were as follows: *Phlebotomus papatasi* (0.26 mm), *P. sergenti* (0.29 mm), *P. alexandri* (0.27 mm), *P. langeroni* (0.25 mm), *P. longicuspis* (0.26 mm), *Phlebotomus* sp. (0.25 mm), *Sergentomyia minuta* (0.20 mm), *S. antennata* (0.24 mm), and *S. clydei* (0.23 mm).

These measurements represent the typical average pharyngeal lengths for males of the examined species, indicating consistent morphometric characteristics across all study sites.

Table 4. Measurements of male pharynx.

Species	Samples per area			Total	Pharynx length (mm)	
	Kalipa	ALRabta	Alqawasim		Mean±SD	Range
P. papatasi	15	0	15	30	0. 26±0.03	0.20-0.30
P. sergenti	15	0	15	30	0. 29±0.04	0.20-0.38
P. alexandri	10	0	0	10	0. 27±0.04	0.22-0.33
P. langeroni	15	9	6	30	0. 25±0.02	0.19-0.27
P. longicuspis	0	13	17	30	0. 26±0.03	0.19-0.31
P. sp.	3	0	2	5	0. 25±0.01	0.24-0.26
S. minuta	12	8	10	30	0. 20±0.02	0.16-0.22
S. antennata	0	0	7	7	0. 24±0.03	0.20-0.26
S. clydei	4	2	0	6	0. 23±0.01	0.22-0.24
F (d.f.)				178	19.0(8,169), P < 0.0001	

Male Aedeagal Measurements

The total lengths of the male aedeagus for the different sand fly species collected from all study areas are presented in Table 5. The recorded mean values were as follows: *Phlebotomus papatasi* (0.14 mm), *P. sergenti* (0.08 mm), *P. alexandri* (0.08 mm), *P. langeroni* (0.17 mm), *P. longicuspis* (0.17 mm), *Phlebotomus* sp. (0.17 mm), *Sergentomyia minuta* (0.13 mm), *S. antennata* (0.14 mm), and *S. clydei* (0.14 mm).

These measurements represent the typical average aedeagus lengths for males of the examined species, reflecting consistent morphometric characteristics across all study sites. Significant differences in male aedeagus length were observed among the species, highlighting its taxonomic and diagnostic value.

Table 5. Measurements of male aedeagus (Mean±SD).

Species	Samples per area			Total	Aedeagus length (mm)	
	Kalipa	ALRabta	Alqawasim		Mean±SD	Range
P. papatasi	15	0	15	30	0.15 ± 0.02	0.11-0.20
P. sergenti	15	0	15	30	0.08 ± 0.005	0.08-0.09
P. alexandri	10	0	0	10	0.08 ± 0.005	0.08-0.09
P. langeroni	15	9	6	30	0.02±0.17	0.13-0.21
P. longicuspis	0	13	17	30	0.01±0.17	0.14-0.20
P. sp	3	0	2	5	0.01±0.17	0.15-0.19
S. minuta	12	8	10	30	0.02±0.13	0.09-0.17
S. antennata	0	0	7	7	0.01±0.14	0.12-0.16
S. clydei	4	2	0	6	0.03±0.14	0.11-0.20
F (d.f.)				**178**	**93.9 (8,169), P < 0.0001**	

Male Coxite Measurements

The total lengths of the male coxite for the different sand fly species collected from all study areas are presented in Table 6. The recorded mean values were as follows: *Phlebotomus papatasi* (0.17 mm), *P. sergenti* (0.28 mm), *P. alexandri* (0.28 mm), *P. langeroni* (0.36 mm), *P. longicuspis* (0.36 mm), *Phlebotomus* sp. (0.39 mm), *Sergentomyia minuta* (0.35 mm), *S. antennata* (0.33 mm), and *S. clydei* (0.35 mm).

These measurements represent the typical average coxite lengths for males of the examined species, indicating consistent morphometric characteristics across all study sites. The results also revealed significant differences in male coxite length among the species, highlighting its importance as a key morphological and taxonomic trait.

Table 6. Measurements of male coxite.

Species	Samples per area			Total	Coxite length (mm)	
	Kalipa	ALRabta	Alqawasim		Mean±SD	Range
P. papatasi	15	0	15	30	0.17 ± 0.005	0.17-0.18
P. sergenti	15	0	15	30	0.23 ± 0.04	0.14-0.31
P. alexandri	10	0	0	10	0.29 ± 0.02	0.26-0.31
P. langeroni	15	9	6	30	0.38 ± 0.03	0.33-0.42
P. longicuspis	0	13	17	30	0.36 ± 0.03	0.31-0.41
P. sp	3	0	2	5	0.39 ± 0.02	0.36-0.42
S. minuta	12	8	10	30	0. 35±0.05	0.29-0.42
S. antennata	0	0	7	7	0.34 ± 0.04	0.29-0.42
S. clydei	4	2	0	6	0.33 ± 0.05	0.21-0.41
F (d.f.)				178	109.7 (8,169), P<0.0001	

Discussion

The present morphometric study revealed statistically significant differences among the examined sand fly species in multiple traits, including antennal lengths, pharyngeal dimensions, and male genital structures (Tables 1–6, $P < 0.0001$ for all measured characters). These significant interspecific differences provide strong evidence for the diagnostic value of classical morphometric characters, confirming their reliability in distinguishing sand fly species. These results are consistent with previous studies in Sudan and North Africa, where Abdalla (2011) and Maríno et al. (2018) reported limited intraspecific variation and clear interspecific differences within Phlebotomus species. Similarly, Hamarsheh et al. (2019) and Almeida et al. (2020) demonstrated that antennal, pharyngeal, and genital traits remain effective for species discrimination, supporting the current findings.

Despite these interspecific differences, the data also demonstrated a high degree of morphological consistency within each species across the three **study sites** (Kalipa, Al Rabta, and Al Qawasim). The measured traits showed limited intraspecific variation, indicating that local populations maintain conserved morphological patterns even in the presence of potential environmental heterogeneity. This stability suggests that the subtle variations observed are likely phenotypic responses rather than fundamental taxonomic divergence.

These observations align with previous morphometric studies that confirmed the effectiveness of classical measurements, such as antennal, pharyngeal, and male genital traits, in discriminating species while showing minimal intraspecific variability (Abdalla, 2011; Maríno et al., 2018; Hamarsheh et al., 2019; Almeida et al., 2020). However, localized morphological variability has been documented under specific ecological pressures, such as variations in genital structures or wing morphology due to environmental gradients (Srinivasan & Jambulingam, 2012; Guilvard & Rioux, 1991; Tesfaye et al., 2025), but the present study shows that such effects are minimal in northwestern Libya.

Overall, the combination of statistically significant differences between species and morphological coherence within species across different sites highlights the robustness of classical morphometry. The interspecific differences serve as reliable diagnostic traits for accurate species identification, while the intraspecific consistency ensures comparability among sympatric populations. This dual pattern of differentiation and stability is particularly valuable for vector surveillance and epidemiological studies, allowing precise species recognition to inform control strategies (Lewis, 1982; Lane, 1993; Killick-Kendrick, 1999; Parvizi & Oshaghi, 2017; Galati et al., 2025; Dokhan et al., 2016; Ready, 2013; Galati, 2018).

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.