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Research article

Using the morphological characters to classify the *Rhopalosiphum Koch*. spp. In Iraqi environments

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ABSTRACT

The morphological features used in the classification of aphid species are supported by microscopic examination. This process requires significant expertise and facing several challenges. The present study aims to collect aphids from different environments and classify them using their morphological features. The different aphid samples were collected during the spring and summertime. The genus and species of aphids were classified. The samples were prepared by cooking the samples in 95% ethanol for 10 min in a nutshell. The body contents of the aphids were boiled for 10–20 min in a 10% KOH solution or for 2–5 min in 40% KOH until they liquefied before being transferred to 5 ml of distilled water. The measurement of the antenna (ANT), the distance along the antenna from the Processus terminalis (ANT PT) tip to the base of the segment I. Base antennal VI: The distance between the basal articulations to the main rhinarium's distal end. The distance between the apical ends of the main rhinarium to the tip of the ultimate antennal segment is known as the processus terminalis (ANT PT or PT). Body length (BL) is the distance between the front's midpoint and the cauda's base. Cauda: The distance between the cauda's center and apex. Siphunculi: the distance between the base and peak of a siphunculi. The length of the ultimate rostral segment (URS) is measured from the tip of the rostrum to the basal articulations of segment 4. HT II: Length of Second segment of hind tarsus from basal articulation to the tip of the segment. According to the above measurement, three species of the genus *Rhopalosiphum* Koch; *Rhopalosiphum maidis, Rhopalosiphum padi, Rhopalosiphum* sp. was identified in the studies area. It can concluded that the morphological feature can be depended on in the classification of aphid but that need to be supported by the other new technologies.

Keywords: Classification, Morphological features, *Rhopalosiphum.*

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1. INTRODUCTION

Aphids, as an important group of insects that belong to the order Homoptera, are very successful creatures with the most species diversity in temperate regions and worldwide distribution [1]. They may cause loss of plants directly or indirectly, plus the direct loss is made by heavy feeding from sap and includes weakness of plant and finally reduction in yield, they cause indirect loss by honeydew secreting on leaves and branches which absorb dust and also mold will start to grow and finally photosynthesis and yield will be reduced. Furthermore, it helps viruses and fungi to infect the plant [2]. Aphids are usually classified in the order Hemiptera, series Sternorryncha along with the psyllids, whiteflies, scale insects, and mealybugs, another approach puts aphids in the order Homoptera and suborder Sternorryncha. Further phylogenetic studies with molecular techniques are in progress there are only eight subfamilies in the family Aphididae [3]. There are many morphological anatomical studies conducted on aphids that provided a background for systematic studies of Aphids. Aphids have a high ability to adaption and change their morphology as a response to enviro-

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nmental factors. Many ecological physiological factors affect the morphology of aphids [4]. The Iraqi area as well as many other nations is plagued by aphids, an economically significant family of insect pests. Nearly 100 species of Aphididae have major economic relevance, and there are about 4700 species worldwide [5]. 450 species have been documented to infest agricultural plants. Their economic importance is primarily attributable to the aphids' ability to reduce crop quality and yield, produce honeydew from their phloem sap-feeding, and spread over 200 plant viruses [6], and feed on plant sap. The *Macrosiphum euphorbiae* potato aphid. There are seven species of the genus Rhopalosiphum Koch that are known to occur in Iraq [7], namely: *R. padi, R. maidis, R. rufiabdominalis, R. prunifoliae, R. insertum, R. lactucae,* and *R. callae*. The present study aims to collect and identify the Rhopalosiphum in different environmental areas using the morphological characteristics.

2. MATERIALS AND METHODS

2.1. Sample collection

Aphid samples were collected for this investigation during a six-month period (November 2014 to April 2015) from a variety of grain crops and grasses in the Iraqi provinces of Baghdad and Diyala. Only apterous viviparous females made up the entire collection of samples. After being placed in plastic bags or containers, the samples were taken to the laboratory, the information gathered at the moment the sample was taken, such as host plant, location, and species details. The sample data are shown in Table 1. The samples were kept at 4 ºC in 90-95 percent ethyl alcohol.

Table 1. The sample information about the *Rhopalosiphum* spp.

No.	Place of collection	Date	Host plant(s)	Common name
A10	Baghdad/Abu Ghraib	25\11\2014	*Sorghum halepense*	Johnson grass
D11	Diyala/ Khan Banisaad	02\12\2014	*Cynodondactylon*.	Bermuda grass
A16	Baghdad/Abu Ghraib	15\12\2014	*Cyperus* sp.	sedges
D2	Diyala/ Khan Banisaad	2\12\2014	*Cynodondactylon*.	Bermuda grass
J5	Baghdad / al-jadiriya	20\11\2014	*Zea mays*	corn
J6	Baghdad / al-jadiriya	23\11\2014	*Loliumrigidum*	tufted grasses
J9	Baghdad / al-jadiriya	23\11\2014	*Sorghum halepense*	Johnson grass
J17	Baghdad / al-jadiriya	13\01\2015	*Cynodondactylon*.	Bermuda grass
A19	Baghdad/Abu Ghraib	15\01\2015	*Triticum*sp.	Wheat
A15	Baghdad/Abu Ghraib	15\01\2015	*Zea mays*	corn
D25	Diyala/ Khan Banisaad	22\01\2015	*Hordeum*sp.	Barley

2.2. Samples preparation

Balsam mounts were prepared using standard procedure. Samples were cooked in 95% ethanol for 10 min in a nutshell. The body contents of the aphids were boiled for 10–20 min in a 10% KOH solution or for 2–5 min in 40% KOH until they liquefied before being transferred to 5 ml of distilled water. The anus and vaginal orifice should show signs of brownish fluid streaming from the body via the needle hole, and they should both be submerged in distilled water for an hour. The aphid was placed in a drop of balsam that had been poured in the middle of the slide. The appendages were then spread out, and the cover slip was gently placed over the specimen. The specimen was then dried for one to two weeks at 50°C. Slides were kept vertically in boxes and drawers from slotted cabinets. Thick card labels attached to the slide with an adhesive substance included collected data. The samples were inspected using a light compound microscope after the microscope had been calibrated (AmScope B490 Compound Binocular Microscope) [8].

2.3. Measurements

Antenna (ANT): The distance along the antenna from the Processus terminalis (ANT PT) tip to the base of segment I. Base antennal VI: The distance between the basal articulation to the main rhinarium's distal end. The distance between the apical ends of the main rhinarium to the tip of the ultimate antennal segment is known as the processus terminalis (ANT PT or PT). Body length (BL) is the distance between the front's midpoint and the cauda's base. Cauda: The distance between the cauda's centre and apex. Siphunculi: the distance between the base and peak of a siphunculi. The length of the ultimate rostral segment (URS) is measured from the tip of the rostrum to the basal articulations of segment 4. HT II: Length of Second segment of hind tarsus from basal articulation to the tip of the segment.

2.4. Treated with species in this study

Identification was done based on apterae. The Current scientific names were taken from the Survey of the World's Aphids [9]. Common names and keys for identifying the collecting samples are taken from Blackman and Eastop, (2006) [10].

3. RESULTS AND DISCUSSION

3.1. Classification and Morphological Characters

Aphids are determined mainly on the basis of their morphological characters and morphometric parameters. Terminology and abbreviations of these characters and parameters in previous references [8, 11]. Table 2 characters used for the identification of aphids in genus and species level, at these levels some other characters may also be useful for identifications, such as the degree of sclerotization, nature of the cuticular surface, the number, size, and shape of hairs or setae, body shape, particular characters of head, thorax, abdomen, and their respective appendages in addition to the biological, anatomical and cytological data [12].

3.2. Subtribe: Rhopalosiphina

The subtribe Rhopalosiphina belongs to the tribe aphidini subfamily Aphidinae family Aphididae until 2001, there were 10 genera in this subtribe worldwide in Iraq there are only four genera (*Hyalopterous*, *Rhopalosiphum*, *Schizaphis* and *Melanaphis*), 2010. This subtribe is distinguished from the

subtribe Aphidina by the Marginal tubercles on abdominal tergum I and VII placed posterio-dorsally to the external spiracles of those targites while in subtribe Aphidina placed posterio-ventrally (Fig. 1).

Table 2. Abbreviation of morphometric parameters characters used in this study.

Abbreviat-ions	Characters
ANT I – VI	Antennomeres: antennal segments I – VI
ANT VI	The terminal antennal segment, usually the sixth but sometimes the fifth, is divided into:
	ANT. VI b: base of antennal segment VI
	ANT PT: processus terminalis of antennal segment VI
BL	The body length is measured from the middle of the frons on the head to the end of the eighth abdominal segment .
Cauda	Posterior appendage at the end of abdomen.
FTC	The Chaetotaxy of first tarsal segment
HT II	The length of the second tarsal segment, or distitarsus, or tarsus 2.
SIPH	Or Cornicle is the structure located on the dorsum of the sixth abdominal segment of aphids
URS	The ultimate rostral segments are the fourth and fifth segments of Rostrum which were treated together.

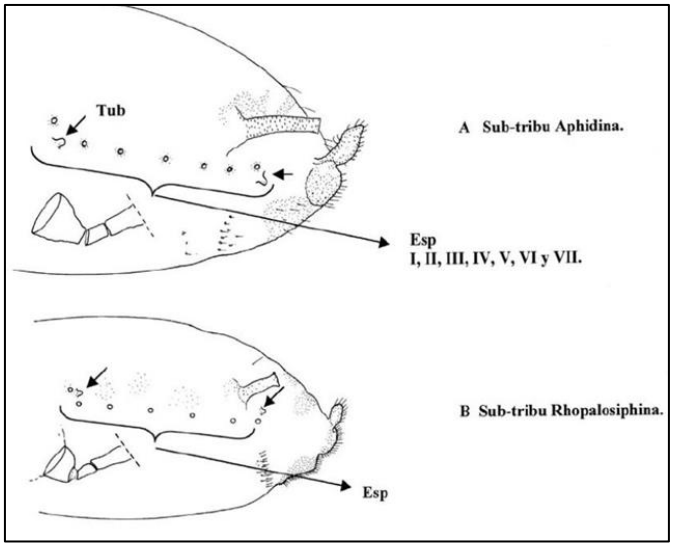

Fig. 1. Differences between subtribe Aphidina and Rhopalosiphina, Tub, marginal tubercle; Esp, external spiracles

Key to genera of the subtribe Rhopalosiphina

1. Siphunculi short and thin, less than 0.05 times as long as Body length and less than 0.5 times as long as cauda; Ultimate rostral segment less than 0.60 times as long as HT II …...…***Hyalopterous*** Koch.
- Siphunculi more than 0.05 times as long as Body length and more than 0.6 times as long as cauda; Ultimate rostral segment less than 0.60 times as long as HT II ……. **2**.
2. Abdominal dorsum with spinules arranged in polygons; Siphunculi cylindrical or tapering for most of length or with very slight swelling of distal part, and with cleared constriction subapically and a well-developed flange……...……*Rhopalosiphum* Koch.
- Abdominal dorsum without spinules; Siphunculi never swelling, and without constriction ……...*Schizaphis* Börnerand *Melanaphis* van der Goot.

3.2.1. Genus *Rhopalosiphum Koch*, 1854

They are small to medium-sized aphids with a body that is broadly oval to rather elongate in shape. Marginal tubercles are normally present on the pronotum and on abdominal segments 1 and 7. The siphunculi are variable in shape - cylindrical, tapering or slightly swollen, always with an apical expansion and a strong flange. The cauda is shorter than the siphunculi and bluntly tapering or tongue shaped (Fig 2). About 15 species associated with Gramineae or Cyperaceae as secondary hosts [13].

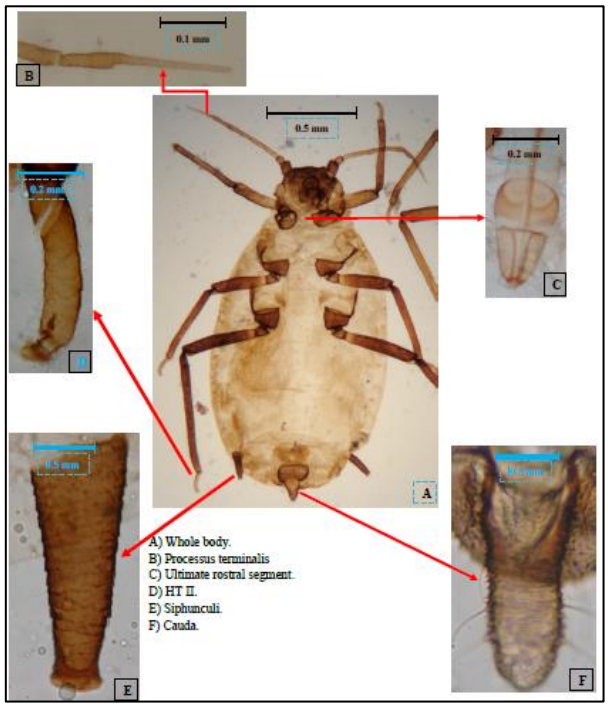

Fig. 2. The many anatomical characters that were used in the classification of *Rh. maidis*.

Key to species of the genus *Rhopalosiphum* spp. In Iraq

1. Body elongate; ANT PT 2.10-2.30 X ANT VI b; URS 0.70-0.92 X HT II; SIPH short and tapering 0.08-0.092 X BL, 1.08-1.30 X cauda which bearing 5-8 hairs……...***Rhopalosiphum maidis***.
- Body broadly oval; ANT PT 3.50-0.4.10 X ANT VI b; URS 1.00-1.20 X HT II; SIPH cylindrical for most of length, with slight distal swelling 0.10-0.34 X BL, 1.49-1.70 X cauda which bearing 4-6 hairs ……..….***Rhopalosiphum padi***.

The present study agreed with all previous local studies on this aphid genus that recorded two species ***Rhopalosiphum maidis***, ***Rh. Padi,*** beside to unknown ***Rhopalosiphum*** sp. that all previous studies in Iraq recorded this species.

Rhopalosiphum maidis

Green Corn Aphid, Barley Aphid, Corn Leaf Aphid
Aphis maidis Fitch, 1856. Ibid. 15: 178-330
Samples No: j2, j5, j6, j9, j10, a19
Apterae rather elongate, with short antennae and, dark siphunculi. The dorsum of the abdomen was yellow-green to dark olive-green or bluish-green, sometimes dusted with wax. In

light-colored specimens head and thorax were dark in contrast to abdominal dorsum. Head dark, antenna 5- or 6-segments, dark in entire length, 0.35-0.44 times as long as body length, processus terminalis (ANT PT) 2.10-2.30 times as long as base antennal VI (ANT VI b). Rostrum hardly reaches up to mid coxae; ultimate rostral segment (URS) was dark, short, 0.70-0.92 times as long as HT II, FTC 3, 3, 2. Abdominal dorsum pale with a net-like pattern and with a pattern of spicules arranged in polygons, sub-genital and anal plate's dark. Siphunculi (SIPH) short, dark, and tapering from the base with only a slight sub-apical constriction and small flange 0.08-0.092 times as long as Body length and 1.08-1.30 times as long as cauda. Cauda tongue-shaped, with similar pigmentation to Siphunculi, **0.06-0.08** times as long as Body length and bearing 4-6 long hairs (Fig 2). Measurements (morphometric parameters) are summarized in Table 3.

Table (3-2) . Morphometric characters (mm) and comparisons of adult morphs of aphids studied sample

Characters	D 25	J 10	D 11	A 16	J 2	J 5	J 6	J 9	J 17	A 19	A 15
BL	(1.85-2.10)	(1.25-1.38)	(1.46-1.57)	(1.43-1.60)	(1.22-1.30)	(1.29-1.67)	(1.25-1.35)	(1.21-1.50)	(1.58-1.68)	(1.85-2.2)	(1.43-1.62)
ANT(I–VI) (length)	(0.700-0.820)	(0.55-0.67)	(0.55-0.66)	((0.60-0.67)	(0.50-0.65)	((0.50-0.62)	(0.50-0.69)	(0.42-0.69)	(0.62-0.68)	(0.70-0.82)	((0.60-0.66)
ANT III (length)	(0.250- 290)	(0.19-0.23)	(0.20-0.25)	(0.16-0.20)	(0.19-0.30)	(0.14-0.27)	(0.19-0.27)	(0.19-0.23)	(0.30-0.35)	(0.16-0.19)	(0.15-0.20)
ANT PT (length)	(0.15-0.18)	(0.10-0.13)	(0.29-0.33)	(0.15-0.17)	(0.15-0.14)	(0.12-0.19)	(0.12-0.15)	(0.13-0.19)	(0.10-0.13)	(0.15-0.18)	(0.15-0.17)
ANT VI b	(0.078-0.092)	(0.06-0.077)	(0.058-0.063)	(0.085-0.098)	(0.05-0.078)	(0.071-0.102)	(0.04-0.079)	(0.04-0.072)	(0.075-0.090)	(0.085-0.095)	(0.088-0.098)
URS (length)	(0.070-0.088)	(0.068-0.86)	(0.058-0.062)	(0.058-0.066)	(0.068-0.85)	(0.043-0.050)	(0.068-0.80)	(0.066-0.89)	(0.088-0.10)	(0.070-0.088)	(0.055-0.065)
URS (accessory hairs)	(2)	(2)	(1)	(2)	(2)	(2)	(2)	(1)	(2)	(2)	(2)
HT II (length)	(0.095-0.11)	(0.11-0.14)	(0.099-0.12)	(0.12-0.13)	(0.12-0.13)	(0.10-0.15)	(0.12-0.15)	(0.12-0.16)	(0.12-0.13)	(0.12-0.15)	(0.12-0.14)
FTC	3,3,2	3,3,2	3,3,2	3,3,2	3,3,2	3,3,2	3,3,2	3,3,2	3,3,3	3,3,2	3,3,2
SIPH (length)	(0.11-0.15)	(0.11-0.13)	(0.11-0.13)	(0.12-0.14)	(0.11-0.12)	(0.10-0.12)	(0.12-0.15)	(0.15-0.16)	(0.18-0.22)	(0.11-0.15)	(0.13-0.15)
Cauda (length)	(0.098-0.12)	(0.10-0.12)	(0.14-0.17)	(0.15-0.17)	(0.12-0.15)	(0.12-0.18)	(0.11-0.15)	(0.11-0.12)	(0.14-0.17)	0.098-0.12)	(0.15-0.18)
Caudal hairs	(12-14)	(4-6)	(6)	(6)	(5-6)	(5-6)	(4-5)	(4-6)	(6-7)	(4-5)	(6-8)
Comparisons ANT(I–VI) / BL	(0.30-0.52)	(0.35-0.44)	(0.42-0.80)	(0.72-0.82)	(0.32-0.48)	(0.70-0.94)	(0.30-0.48)	(0.31-0.48)	(0.70-0.82)	(0.33-0.48)	(0.73-0.99)
ANT PT / ANT VI b	(1.90-2.20)	(2.10-2.30)	(3.50-0.4.10)	(3.90-0.4.20)	(2.09-2.22)	(3.97-0.4.80)	(2.12-2.34)	(2.2-2.38)	(3.90-0.4.44)	(2.20-2.50)	(3.40-0.4.60)
URS / HT II	(0.71-0.88)	(0.70-0.92)	(1.00-1.20)	(1.00-1.16)	(0.73-0.90)	(1.04-1.18)	(0.77-0.98)	(0.77-0.97)	(0.70-1.16)	(0.77-1.04)	(0.40-1.40)
SIPH / BL	(0.050-0.076)	(0.08-0.092)	(0.10-0.34)	(0.12-0.19)	(0.08-0.090)	(0.11-0.12)	(0.05-0.097)	(0.06-0.090)	(0.09-0.30)	(0.03-0.087)	(0.03-0.37)
SIPH / Cauda	(1.12-1.26)	(1.08-1.30)	(1.49-1.70)	(1.80-1.99)	(1.10-1.32)	(1.80-1.99)	(1.00-1.37)	(1.04-1.30)	(2.10-2.70)	(1.04-1.39)	(2.15-2.90)
Cauda / BL	(0.43-0.62)	(0.06-0.08)	(0.040-0.087)	(0.088-0.097)	(0.05-0.09)	(0.077-0.109)	(0.04-0.09)	(0.02-0.06)	(0.060-0.120)	(0.01-0.12)	(0.060-0.120)

Rhopalosiphum padi

Bird Cherry-Oat Aphid, ***Aphis padi***Linnaeus, 1758. Syst. Nat., ed. 10: 451, Samples No: d11, a15, a16, j17.

Apterae on grasses and cereals, broadly oval, green mottled with yellowish-green or olive-green, or dark-olive to greenish black; often with rust-colored patches around the bases of siphunculi. These patches sometimes meet on the abdominal dorsum. Head smooth and dark, antenna 6 segments, dark in entire length, 0.42-0.80 times as long as body length, processes terminalis (ANT PT) 3.50-0.4.10 times as long as base antennal VI (ANT VI b). Rostrum reaches mid coxae; ultimate rostral segment (URS) dark, short, with a broad base about **1.00-1.20** times as long as HT II, FTC 3, 3, 2. The abdominal dorsum is pale with a net-like pattern and affiant net-like pattern and with a pattern of spicules arranged in polygons, sub-genital and anal plate dark. Siphunculi (SIPH) dark, cylindrical for most of the length, with slight distal swelling, and with a marked subapical constriction and large flange 0.10-0.34 times as long as Body length and **1.49-1.70** times as long as cauda. Cauda is tongue-shaped, with similar pigmentation to Siphunculi, **0.040-0.087** times as long as Body length and bearing 5-8 long hairs, fig. 3.

Rhopalosiphum sp.

Samples No: d 25. Dark green or olive with usually a reddish area at the posterior end of the abdomen between and around Siphunculi, head smooth and dark, antenna 6-segments, dark in entire length, 0.30-0.52 times as long as body length, processus terminalis (ANT PT) 1.90-2.20 times as long as base antennal VI (ANT VI b).

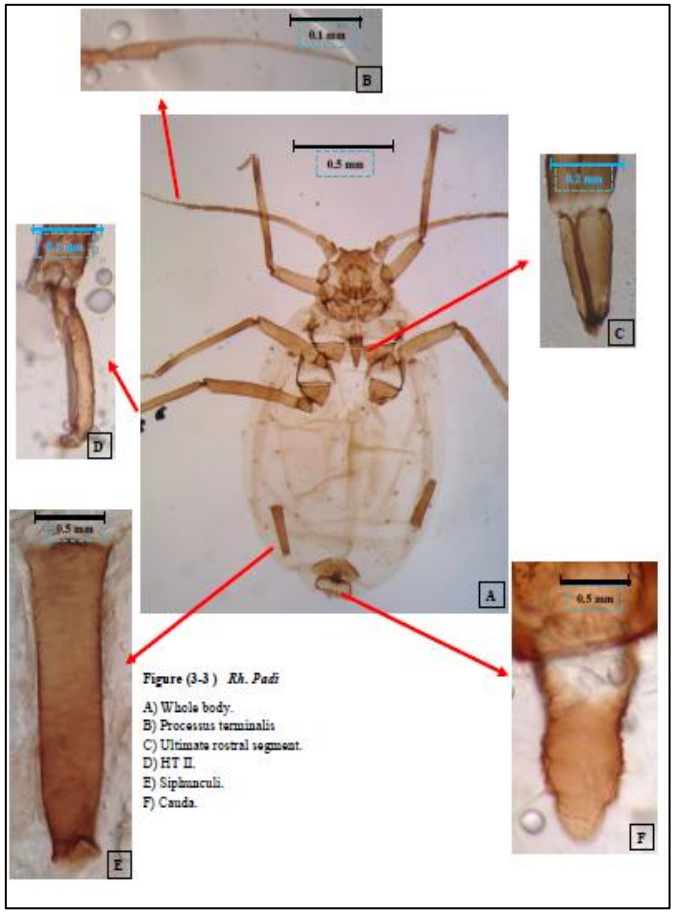

Fig. 3. The many anatomical characters that were used in the classification of *Rh. Padi*

Rostrum hardly reaches mid coxae; ultimate rostral segment (URS) is dark, short, about 0.71-0.88 times as long as HT II, FTC 3, 3, 2. Abdominal dorsum pale, sub-genital, and anal plate dark. Siphunculi (SIPH) were dark, and cylindrical for most of the length, tapering from the base with small clear flange 0.09-0.30 times as long as body length and 2.10-2.70 times as long as cauda. Cauda tongue-shaped, with lighter pigmentation to Siphunculi, 0.060-0.120 times as long as Body length and bearing (4- 6) long hairs (Fig. 4). Measurements (morphometric parameters) are summarized in Table 3.

Despite the significant amount of study done on them and the economic significance of many Rhopalosiphum species, it can be challenging to tell one species from another. This study uses morphological characteristics to more accurately characterize and distinguish between Rhopalosiphum padi and R. insertum. The utility of this characteristic in distinguishing between these two species is illustrated by bivariate plots of the length of the antennal flagellum against the length of the body, length of the hind tibia, and length of antennal segment III. There are provided discriminant functions for apterae and alatae of these two species. It was discovered that there was little morphological modification in either species in response to temperature and host changes. Both species' karyotypes were utilized to authenticate their identities, and the intraspecific chromosomal variability was investigated [14].

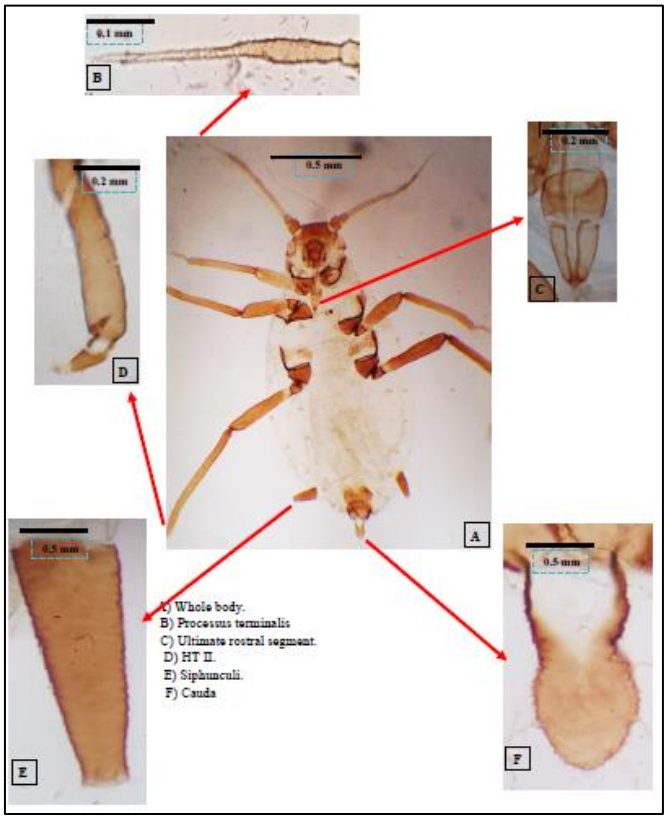

Fig. 4. The many anatomical characters that were used in the classification of *Rhopalosiphum sp.*

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the Ethical Committee of the University of Baghdad, Baghdad, Iraq (No 934, 2020).

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