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Morphological characters and feeding habits of insectivorous Kuhl's pipistrelle bat *Pipistrellus kuhlii* Cretzschmar, 1830, in different riverine habitats

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ABSTRACT

This study revises the morphological characters of both sexes of Kuhl's pipistrelle, including external, cranial and dental characters. This study also establishes a baseline for the diet of this insectivorous bat species across a variety of habitats in the Nile Valley, where diets have never before been investigated. Our findings of the stomach contents were not significantly affected despite the different habitats from which the samples were collected. The three most common insect taxa recovered were Hymenoptera, Lepidoptera and Coleoptera. The percentage volume of insect orders in the diet of Kuhl's bat (63 stomachs) consisted of 29% of Hymenoptera, 9% of Coleoptera, 27% of Lepidoptera, 32% unknown materials and unexpected thing of these insectivorous bats was the presences of 3% fruits. A single order did not dominate the diet of this specie because Hymenoptera was the most fed insect orders by frequency of occurrence 87.3%, Lepidoptera by 74.6%, Coleoptera 36.5% and fruits in 4.8% of samples. Also, they may also be fed by other insect orders, as unknown materials were found in all samples.

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1. Introduction

Bats (Chiroptera) are one of the most diverse and species-rich terrestrial vertebrate orders on the planet because they live in almost all environments, from rainforests to deserts, and all in between, ranging from subarctic to tropical latitudes as well as they use a variety of foraging strategies, social habits, vocal communication, navigational tools and learning abilities (Amichai, 2017).

Egypt's mammalian fauna has been studied since the Linnaean era, and many species of mammals, including bats, have now been studied and described. Bats have been the subject of several studies over the last two centuries, according to (Benda et al. 2008), and expertise in the fields of taxonomy, ecology, and species distribution has evolved over time.. Osborn and Helmy (1980) describe Egypt's mammalian fauna, which included Egyptian bats from all over Egypt, including Nubia and Sinai, following that, many research on

bats. The most reliable summary of Egyptian bat records was Qumsiyeh's (1985) review; then Harrison and Bates (1991) book "Mammals of Arabia" which included bat taxonomy, ecology, and species distribution in Arabia. Wassif, 1995 paper describe many Egyptian bats from all over Egypt, including Sinai, and the identification key of Egyptian bats by (Dietz, 2005), while the most recent paper on bats was published by (Benda et al. 2008).

Until now, bat research in this region has lacked information regarding the biology of the bat species occurring in Egypt, and there is no available information on diet. Here, we focus on Kuhl's pipistrelle, *Pipistrellus kuhlii*, it's a member of family Vespertilionidae and it's one of the most common and abundant bat species distributed in the Mediterranean basin of the Palaearctic region, and Middle East (Bray et al. 2013; Dalhoumi et al. 2018). Its range covers most of the Arabian Peninsula and extends to southwestern Asia in East (Sharifi et al. 2004). The distribution of the Kuhl's pipistrelle bat is expanding in Northern Africa and in the Middle East (Bar-David et al. 2015). It occupies mainly lowlands, coastal areas, river valleys, but also urban areas such as towns and suburbs (Qumsiyeh, 1985; Mendelsohn and Yom-Tov 1999; Benda et al. 2006; WaWrocka et al., 2012). Due to this expansion in distribution range, *P. kuhlii* can be recognized as an adaptable generalist that easily adapted in urbanized environments (Russo and Ancillotto 2015; Ancillotto et al. 2015), this expansion show

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that Kuhl's pipistrelle bat is probably behaviorally and physiologically adept to adjusting climate conditions outside its optimal range (Amichai and Korine 2020).

The knowledge of an animal's diet is critical for understanding its ecological function as prey or as a predator, and its effect on local ecosystems. Since natural habitats are being altered as a result of increased urbanization and modern agricultural practices, this understanding is particularly important (Whitker et al. 1994). According to WaWrocka et al. (2012) the ability of animals to successfully forage is affected by the abundance and availability of their prey. Prey abundance (frequency and distribution in the environment), for insectivorous Kuhl's bat has been defined as a selective opportunist whereas it doesn't show prefer a specific insects or feeding habitat, it tends to feed on insect-rich patches and to prefer larger prey items over smaller ones according to prey availability (Goiti et al. 2003). So, the diet composition of this bat can differ between seasons and habitats for a variety of reasons, including weather, prey availability, and population cycles.

This small bat species considered as a generalist insectivore, and its high behavioral flexibility, in much of its range, may utilize variety of cluttered environments (Kalko and Schnitzler 1993). It has been detected hunting over open natural land and agricultural fields, above water ponds, at vegetation edge and attracted to insects on street lights in rural and urban areas (Kalko and Schnitzler 1993; Ancillotto et al. 2015, 2016; Kahnonitch et al. 2018).

2. Materials and methods

2.1. Sampling

Pipistrelle bat Samples were collected from 10 roosting and foraging sites (n = 63, nearly 6 per location) collected from different habitats, which represented agricultural lands, fields, and densely populated urban areas in the governorates of Beni Suef and Minya Fig. 1. These habitats were visited in the Middle Nile Valley in

Egypt, an area characterized by the narrow flood plain (fertile areas cultivated on both sides of the Nile River) and bounded on the eastern and western sides by vast desert. Mist nets were used to catch bats, according to the Indiana Bat Protocol (USFWS, 1999). Net sets were installed over water sources or near edge habitats, as well as in interior agriculture fields, according to (Carroll et al. 2002; Feldhamer et al. 2001). For each individual bat captured, its species, gender and body mass were recorded.

2.2. Morphological analysis

All morphological characters used in this study is a descriptive data for species identification and its including: Eight external measurements were taken for each individual bat using a 0.1 mm caliper, according to (Benda et al. 2008). These were; **FA** = Forearm length, **D5** = 5th Digit length, **D3** = 3rd Digit length, **TBL** = Total Body Length, **TL** = Tail Length, **WS** = Wing Span (width of the bat), **EL** = Ear Length and **HF** = Hind Foot length. The ninth external character measured was **BM** = Body Mass in grams (g). And eight cranial measurements were taken for each skull using a 0.1 mm caliper, according to (Benda et al. 2008). The measurements were; **GLS** = Greatest Length of Skull, **CBL** = Condylbasal Length, **CCL** = Condylcanine Length, **ZB** = Zygomatic Breadth, **SL** = Snout Length, **BL** = Bulla Length, **BW** = Bulla Width and **RWC** = Width of Canine Row. An additional twelve dental measurements were taken for each bat's jaw by using a 0.1 mm caliper. **IM³** = length of upper tooth-row between I¹ and M³, **CM²** = length of upper tooth-row between C and M², **CM³** = length of upper tooth-row between C and M³, **M¹M³** = length of upper molars-row between M¹ and M³, **CPm¹** = length of upper tooth-row between C and pm¹, **CML** = Condylar Mandible Length, **CPH** = Coronoid Process Height, **IM₃** = length of lower tooth-row between I₁ and M₃, **CM₃** = length of lower tooth-row between C and M₃, **M₁M₃** = length of lower molars-row between M₁ and M₃, **CPm₂** = length of lower tooth-row between C and pm₂ and **CPm₁** = length of lower tooth-row between C and pm₁.

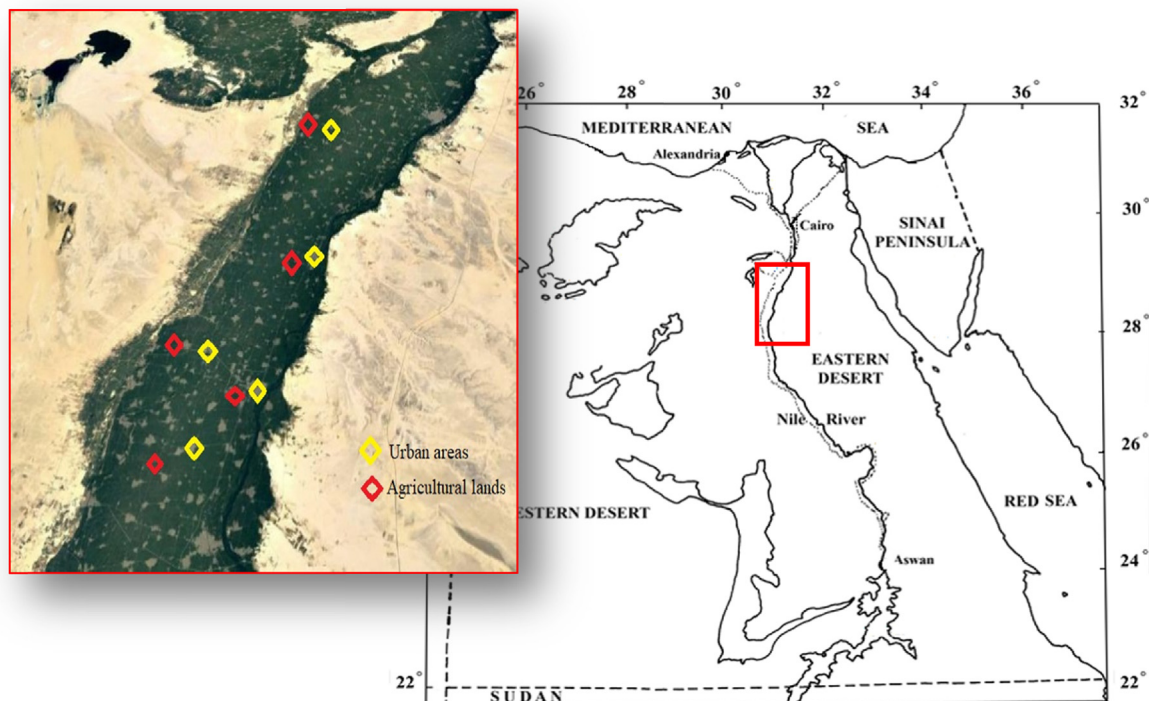


Fig. 1. A map showing Study Area and Collection Localities of Kuhl's pipistrelle bat samples.

2.3. Stomach contents analysis

Whole stomachs and their contents were removed from bats while they were freshly dead. Stomach contents were weighed to

the nearest 0.1 mg and preserved in 70 % ethanol before analysis. Contents were analyzed in the laboratory to determine their food components and the percent volume of different dietary items.

Undigested prey remains (e.g., invertebrate exoskeletons, heads, antennae, mouthparts, prothoraxes, pterothoraxes, wings, legs, and other fragments) were compared to a reference collection to identify food items. All parts were examined under a stereoscopic microscope. By comparing parts to whole specimens, all insect remains were identified to order level using a variety of keys (Whitker, 1988; 2004; McAney et al. 1991; Borror et al. 1989). The value of each prey species in the diet was determined by its frequency (F) of occurrence (percentage of samples in which a given prey species was found) (Karanth and Sunquist 1995; Nunez et al. 2000; Taber et al. 1997).

3. Results

3.1. Descriptive morphological characters

External morphology is widely used as descriptive data to distinguish bat species as well as to determine differences between individuals of the same species of both sexes. Based on the external characters taken for each individual of both sexes of Kuhl's pipistrelle bats (Fig. 2), total body length ranged between 60.3 and 76.4 mm, and tail length: 21.4–36.4 mm, width of the bat (wings length): 118–175 mm, the forearm length: 32–34.6 mm, 5th digit: 40–44.5 mm, and 3rd digit: 54–61 mm. The weight of Kuhl's pipistrelle bats ranged from 4.5 to 5.8 gm. Pelage dense, fine and short, fur color were very variable but were usually dark, ventral hairs with white tips and black bases whereas the dorsal hairs were buff



Fig. 2. The preserved skin of Kuhl's pipistrelle bat.



Fig. 3. The skull of Kuhl's pipistrelle bat, A) ventral view, B) lateral view, C) occipital view and D) lower jaw.

tipped and had deep black bases, and naked parts of skin dark brown to blackish. Body color was variable with habitats, and their ears lengths ranged between 8.2 and 9.6 mm. Upper edge was narrow, front margin was nearly erect and the upper tooth row length CM3 was more than 4.5 mm.

Based on the external characters of Kuhl's pipistrelle bats, the male individuals of this species are slightly larger than females in most external features (Annex 1). The mean weight of males was 5.2 g and ranged between 4.9 and 5.8 g while the mean weight of females was 4.8 g and ranged between 4.5 and 5.4 g. The mean

body length of males (TBL) was 166.3 mm and ranged between 152 and 175 mm while the mean width of females was 149.5 mm and ranged between 118 and 168 mm. The mean width of males (WS) was 166.3 mm and ranged between 152 and 175 mm while the mean width of females was 149.5 mm and ranged between 118 and 168 mm. The mean forearm length of males was 33.2 mm, ranging between 32 and 34.6 mm while the mean forearm length of females was 32.9 mm, ranging between 32.4 and 33.3 mm. The mean length of the 5th digit of males was 41.9 mm, ranging between 40 and 44.5 mm while the mean length of the 5th digit

Table 1
Number of occurrence and percentage of Frequency (F) and Volume (V) of food items in the stomach contents of *Kuhl's pipistrelle* bat samples from each habitats.

Food Items	Agricultural lands			Urban areas		
	No	Frequency (F)	Volume (V)	No	Frequency (F)	Volume (V)
Hymenoptera	30	88 %	30 %	25	86.2 %	29 %
Coleoptera	12	35.2 %	10 %	11	38 %	8 %
Lepidoptera	25	73.5 %	27 %	22	75.9 %	25 %
Fruits	-	0 %	0 %	3	10.3 %	8 %
Unknown materials	34	100 %	33 %	29	100 %	30 %

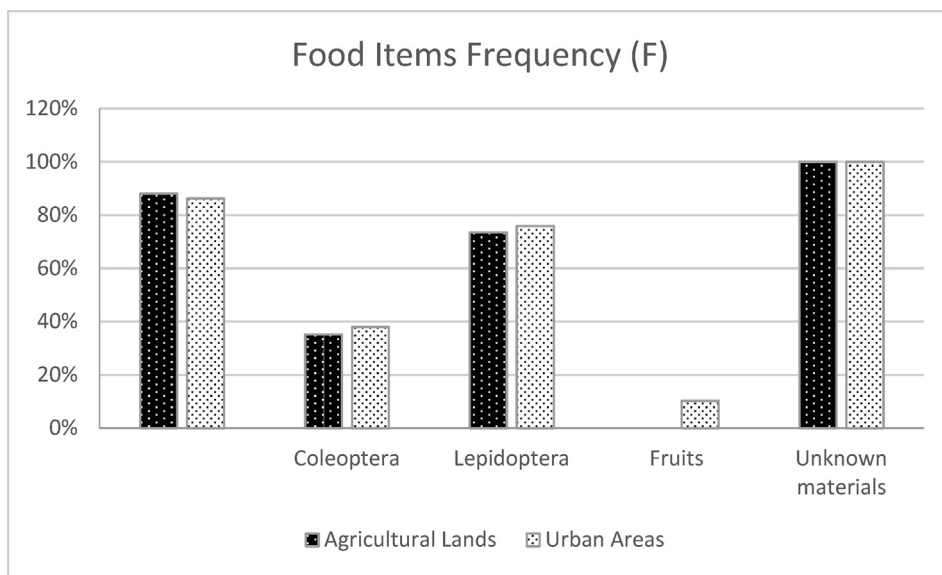


Fig. 4. Percentage of the Frequency occurrence (F) of food items in the stomach contents of Kuhl's pipistrelle bat samples from each habitats.

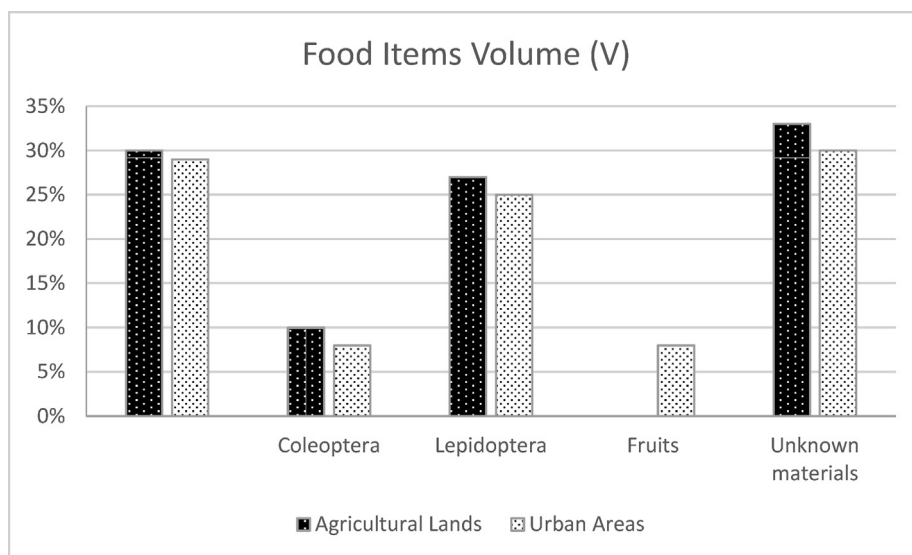


Fig. 5. Percentage of the Volume (V) of food items in the stomach contents of Kuhl's pipistrelle bat samples from each habitats.

of females was 40.5 mm and ranged between 40 and 41.4 mm. The mean length of the 3rd digit of males was 56.2 mm and ranged between 54 and 58.6 mm while the mean length of the 3rd digit of females was 55.1 mm and ranged between 54.1 and 57.2 mm. The mean tail length of males was 30.7 mm and ranged between 27.5 and 36.4 mm while the mean tail length of females was 27.8 mm and ranged between 21.4 and 31.5 mm. One exception from the above results of external characters was the finding that ear length and hind foot length were slightly larger in females than in males (Annex 1) (see Fig. 3).

Nevertheless, the cranial and dental characters were taken for individuals of both sexes of Kuhl's pipistrelle bats showed that, the males and females of this species were shown to be nearly equal in most cranial and dental features (Fig. 3).

3.2. Food and feeding habits

Our microscopic examination of the stomach content samples from each habitats, showed that, the general composition of the bat's diet in agricultural lands were the Hymenoptera which showed the highest frequency (F) of occurrence of all insect orders in the diet, found in 30 stomachs equal 88 % of 34 bats samples were collected from agricultural lands, the volume of insects of this order in the diet of Kuhl's bat was 30 %, while the prey species belonging to Lepidoptera were the second most frequently recognized insect order, found in 25 stomachs and 73.5 % of all bat samples, which represented 27 % of the total volume. Coleoptera were the third detected prey of all the insect orders, present in the stomachs of 12 bats and 35.2 % of all bats samples, representing 10 % of the volume of the bat's diet in agricultural lands. Kuhl's bat species may also feed on other insect orders, not identified here, because

Table 2
Number of occurrence and percentage of Frequency (F) and Volume (V) of food items in the stomach contents of all Kuhl's pipistrelle bat samples.

Food Items	no	Frequency (F)	Volume (V)
Hymenoptera	55	87 %	29 %
Coleoptera	23	36.50 %	9 %
Lepidoptera	47	74.60 %	27 %
Fruits	3	4.80 %	3 %
Unknown materials	63	100 %	32 %

the presence of unknown digested materials in all samples which equal 33 % of the volume of stomach contents (Table 1 and Figs. 4 and 5).

On the other hand, the general composition of the bat's diet in the urban areas were the Hymenoptera which showed the highest frequency (F) of occurrence of all insect orders in the diet, found in 25 stomachs equal 86.2 % of 29 bats samples were collected from the urban areas, the volume of insects of this order in the diet of Kuhl's bat was 29 %, while the prey species belonging to Lepidoptera were the second most frequently recognized insect order, found in 22 stomachs and 75.9 % of all bat samples in this habitats, which represented 25 % of the total volume. Coleoptera were the third detected prey of all the insect orders, present in the stomachs of 11 bats and 38 % of all bats samples, representing 8 % of the volume of the bat's diet in the urban areas. One of the most surprising result was the presence of fruits remains in three samples, equaling 10.3 % of the bats sampled in the urban areas and representing 8 % of the volume of stomach contents on average. Also, we cannot be certain that this species does not feed on other insect orders, not identified here, because the presence of unknown digested materials in all samples which equal 30 % of the volume of stomach contents (Table 1 and Figs. 4 and 5).

Through the microscopic examination of the stomach content samples in above results, we found that, despite the different habitats from which the samples were collected, they did not significantly affect the type of insects that were identified in the stomach contents at the level of insect order. There may have been differences at the level of the insect species within the same order. However, the species-level identification of insects from stomach contents is very difficult because specimens are often partially digested. As a result, the dietary findings at the order level were nearly consistent across different habitats.

The results of the stomach content samples from both habitats in (Table 2; Figs. 6 and 7), showed that, the general composition of the bat's diet, expressed as the number of stomachs containing the food item, its frequency (F) of occurrence (its percentage of all samples) and the volume of all food items found.

According to our finding Hymenoptera showed the highest frequency (F) of occurrence of all insect orders in the diet, found in 25 stomachs and 87.3 % of all bats sampled under this study, the percentage volume of insects of this order in the diet of Kuhl's bat was 29 %. While the prey species belonging to Lepidoptera were the

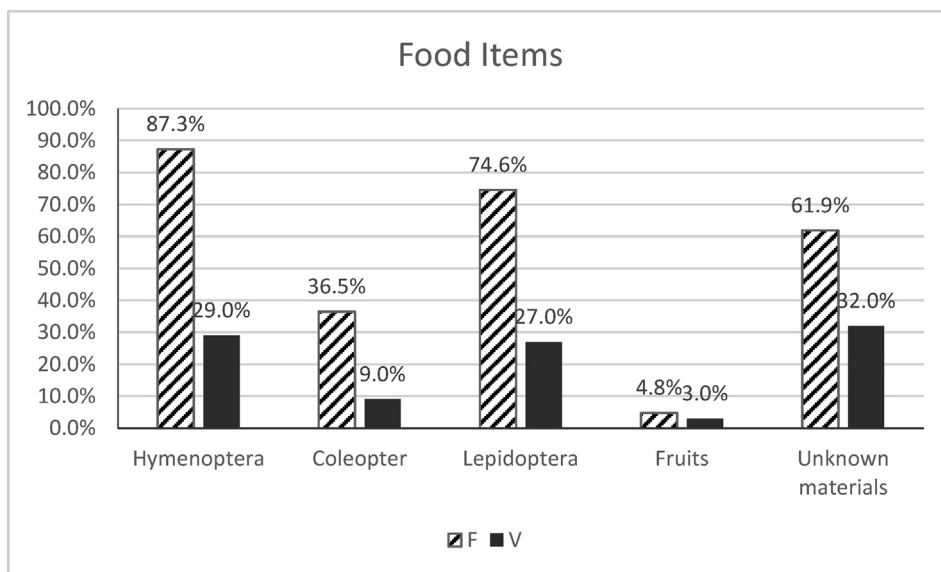


Fig. 6. Percentage of the Frequency occurrence (F) and Volume (V) of food items in the stomach contents of Kuhl's pipistrelle bat samples.

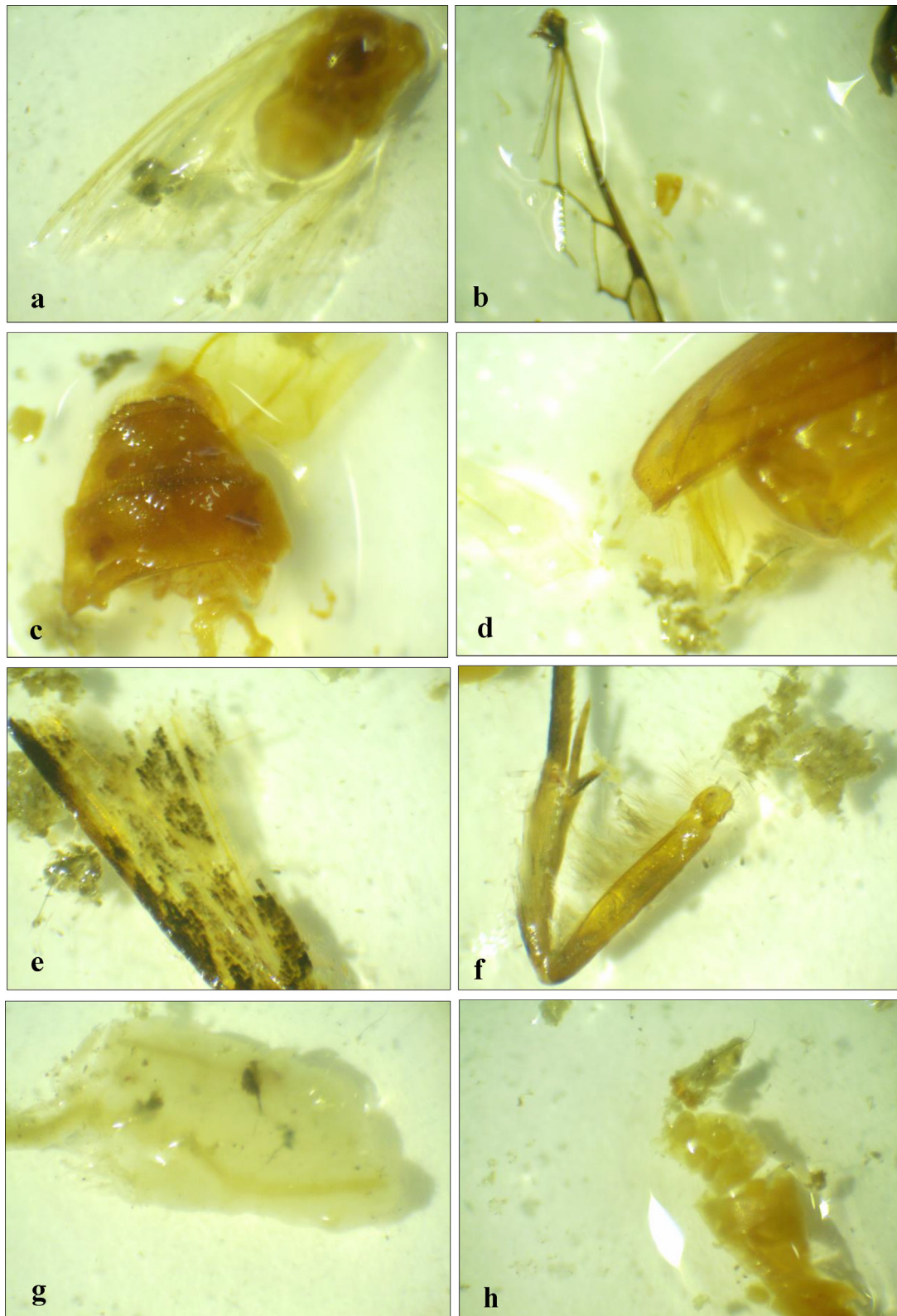


Fig. 7. Some insect's parts and another food items under microscope a,b) represented Hymenopteran parts, c,d) Coleopteran parts, e,f) Lepidoptera insects parts and g,h) represented the fruit remains in the stomach contents of Kuhl's pipistrelle bat samples.

second most frequently detected insect order, found in 47 stomachs and 74.6 % of all bat samples. The percentage volume of Lepidopteran insects in the diet of Kuhl's bat was 27 %. Coleoptera were the third most frequently detected prey of all the insect orders,

present in the stomachs of 23 bats and 36.5 % of all bats samples, representing 9 % of the volume of insects in the diet. Kuhl's bat species may also feed on other insect orders, not identified here, because the presence of unknown digested materials in all samples

which equal 32 % of the volume of stomach contents (Table 2; Figs. 6 and 7).

These results reveal that the diet composition of the Kuhl's bat species varies widely, and not dominated by a single order of insects, but rather than this species prefers the species of some insect orders more than others. Also this may be due to the abundance of different prey orders and their availability as prey during the main foraging activity times of this small bat. One of the most surprising result was the presence of fruits in three samples, equaling 4.8 % of the bats sampled in our study and representing 3 % of the volume of stomach contents on average. Consuming fruit may be a result of an adaptation by these insectivorous bats living in urban areas. It may be some bats favored foraging on insects above fruit remains in the trash, or alternatively, bats may be collecting insects from nearby fruit gardens.

4. Discussion

According to our descriptive morphological findings, we confirmed that the samples collected in the field belong to *Pipistrellus kuhlii*, as all measurements were compatible and within the limits of previous studies to identify this species, such as Qumsiyeh (1985) and the most recent identification key of Egyptian bats by (Dietz, 2005) and compatible with many measurements were taken from Europe and Eastern Mediterranean (Mendelssohn and Yom-Tov 1999; Dietz and von Helversen 2004; Benda et al. 2006). Whereas the *Pipistrellus kuhlii* upper tooth row length (CM3) must be greater than 4.5 mm according to Egyptian key, our results range from 4.5 mm to 4.9 mm, according to Egyptian key and Europe and Eastern Mediterranean key respectively, forearm length (FA) must be 30.7 – 37.4 mm and 31–40 mm, our results range from 32 mm to 34.6 mm, length of 5th digit (D5) was 40 – 45 mm and 40–45 mm, our results range from 40 mm to 44.5 mm, and length of 3rd digit (D3) was 54 – 61 mm and 54–61 mm, our results range between 54 mm and 58.6 mm. On the other hand, some measurements show a slightly difference with many measurements were taken after (Mendelssohn and Yom-Tov 1999; Dietz and von Helversen 2004; Benda et al. 2006) as in Greater length of skull range between 12.3 and 13.9 mm, our results range between 12.4 mm and 12.9 mm and Zygomatic breadth range between 7.8 and 9.3 mm, our results range between 7.0 mm and 7.4 mm. Also, by comparing the measurements of external and morphological characteristics, it was revealed that the male individuals of Kuhl's pipistrelle bats are slightly larger than females with regards to most external features but are nearly identical in most cranial and dental characters.

The majority of published data on the food habits of bats are based on analyses of stomach and fecal contents. Stomach contents analyses allow examination of the undigested items of the last meal, whereas fecal analysis is biased towards detection of less digestible items because digestion appears to degrade soft parts quickly (Whitaker, 1988). Therefore, to obtain the best results, the stomach content analysis method was used in this study. According to our diet composition results despite the different habitats from which the samples were collected, they did not significantly affect the type of insects that were identified in the stomach contents at the level of insect order. There may have been differences at the level of the insect species within the same order, this may be due to the overlap of these habitats and their close proximity to each other, or it may be due to the nature of Kuhl's bats and their ability to forage over variety of habitats as open natural land and agricultural fields, above water ponds, at vegetation edge and attracted to insects on street lights in rural and urban areas (Kalko and Schnitzler 1993; Ancillotto et al. 2015, 2016; Kahnonitch et al. 2018). Kuhl's bat has been considered as a gener-

alist insectivore, with high behavioral flexibility, may utilize variety of environments (Kalko and Schnitzler 1993), whereas it doesn't show prefer a specific insects or feeding habitat (Goiti et al. 2003). According to our finding, prey species belonging to the insect order Hymenoptera had the highest frequency of occurrence and are thus on the top of the food spectrum of the *Pipistrellus kuhlii* bats, followed by Lepidoptera and Coleoptera sequentially. This insects orders were consistent with available information on the diet of *Pipistrellus* bats, including Coleoptera, Diptera, Hemiptera, Heteroptera, Hymenoptera, and Lepidoptera were reported after (Whitaker et al. 1994; Feldman et al. 2000; Goiti et al. 2003; Benda et al. 2006, 2010; Cohen et al. 2020). As previous studies indicate that, the variation in the volumetric representation of insect orders were preyed by this bat depending on the variation of the geographical location and the seasons, Whitaker et al. (1994), found that *Pipistrellus kuhlii* in one location fed mainly on hymenopterans and it's make up 56 %, followed by Coleoptera (32 %) and Lepidoptera (6 %). While in another location Diptera was a dominant group (31 %), followed by Lepidoptera (24 %), Coleoptera (17 %), and Hymenoptera (15 %) (Feldman et al. 2000). In Syria Benda et al. (2006) found that Hemiptera was a dominant group (35 %), followed by Coleoptera (21 %), Hymenoptera (14 %), and Lepidoptera (11 %). The variability of the frequency of some insect species belonging to one order compared to another may be due to the generalist behavior and high behavioral adaptability, of this bat species. So, the more likely reason is that it is due to the availability and abundance of these prey orders in foraging areas, Whitaker and Karataş (2009) concluded that *Pipistrellus* bats feeding on a variety of insect species and can be treated as generalists. Fruits and unknown materials were also found to be present in the stomachs of this bat species.

While *Pipistrellus kuhlii* bats are historically known for their feeding habits that focus on a small number of insect orders, consumed at high frequency and volume in their diet, our findings show that they feed on a variety of insect orders. As a result, insects are an essential source of energy for bats. *Pipistrellus kuhlii* bats feed mainly in the first few hours after sunset, and their feeding behavior is characterized by low, quick feeding flights in open areas (Barak and Yom-Tov 1989). According to Serangeli et al. (2012) and Maxinová et al. (2016) the home range of Kuhl's bat is less than 2 km² but the foraging sites as far as 4.5 km.

While fruit remains occurred in the diet, they were not represented by a high frequency of occurrence or volume in the stomach and remains were restricted to three individuals which collected from the urban areas. This suggests that these were consumed accidentally by bats or alternatively it could be a result of an adapted feature by these insectivorous bats as a result of living in urban areas. Bats might forage on insects flying or attached to fruit remains in trash. For example, this bat was attracted to insects swarming around ripe fruits on a peach tree in East Africa, according to Kingdon (1974), which may explain the presence of fruits remains in their diet.

5. Conclusion

This study also establishes a baseline for the diet of this insectivorous bat species across a variety of habitats in the Nile Valley. Our findings of the stomach contents, despite the different habitats from which the samples were collected, didn't significantly affect the type of insects that were identified at the level of insect order. Still, there may have been differences at the level of the insect species within the same order. The three most common insect taxa recovered were Hymenoptera, Lepidoptera and Coleoptera. The percentage volume of insect orders in the diet of Kuhl's bat (63 stomachs) consisted of 29 % of Hymenoptera, 9 % of Coleoptera,

27 % of Lepidoptera, 32 % unknown materials and unexpected thing of these insectivorous bats was the presences of 3 % fruits. A single order did not dominate the diet of this specie because Hymenoptera was the most fed insect orders by frequency of occurrence 87.3 %, Lepidoptera by 74.6 %, Coleoptera 36.5 % and fruits in 4.8 % of samples. Also, they may be fed by other insect orders, as unknown materials were found in all samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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