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Mapping of anopheles mosquitoes (Diptera: Culicidae) in EnNuhud Town, West Kordofan state, Sudan

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Abstract
Climatic changes, especially rainfall, affect the distribution and densities of mosquitoes. This study was carried out to determine mosquito species composition and mosquito density, in addition to describing mosquito aquatic habitats in EnNuhud Town, capital of West Kordofan State, Sudan. A cross-sectional survey of Anopheline mosquito larval habitats was conducted in EnNahud Town (8 blocks/ sentinel sites) during July 2014 to June 2015. Larvae were collected using the standard dipping technique; while adults were collected using pyrethrum-sprayed sheet method. The habitats observed were broken pipes, bricks traditional factories (Kaminas), pools and swamps formed after rainfall. A total of 2,482 Anopheline mosquito larvae were collected. Of these, 2,449 (98.7%) were morphologically identified as *Anopheles gambiae s.l* , 33 (1.3%) as *A. rufipes*. *A. arabiensis* was found in all blocks. While *A. rufipes*, was found in one block (Algady block). The high mosquito larval densities indicated that EnNahud Town inhabitants are at risk of mosquito-borne diseases, including malaria. Therefore, malaria control program targeting both the immatures and adults should be enhanced, especially during the rainy-season.

Keywords: Mosquitoes, *Anopheles*, mapping, West Kordofan state, Sudan

1. Introduction

Mosquito-borne diseases, especially malaria, remain the leading health problem. It is estimated that at least 500 million people suffer from mosquito-borne diseases and more than 1.1 million people die of malaria and dengue annually annually [1]. These diseases have accounted for huge economic loss, mortality, low productivity and social discrimination in many developing countries and to a significant health burden in developed countries via travelers who have not taken sufficient precautions and prophylactic medications before travelling [2].

Human malaria parasites are transmitted by mosquitoes of the genus *Anopheles* and their geographic distribution is the result of a complex interaction of biogeography, including biotic (*e.g.* competition and dispersal) and a biotic factors (*e.g*. climate and topography) that can vary in both time and space [3]. Africa has over 140 recorded *Anopheles* species, of which at least eight are considered to be effective vectors of malaria. Two of the most efficient vectors of human malaria, *Anopheles gambiae sensu stricto* (hereafter *A. gambiae*) and *A. arabiensis* are members of the *A. gambiae* complex [4]. Other recognized species of the complex are *A. merus*, *A. melas*, *A. quadriannulatus*, *A. quadriannulatus* B, and *A. bwambae*. *A. merus* and *A. melas* are associated with salt-water with a localized distribution along the eastern and western coasts of Africa, respectively, while *A. bwambae* has only been found breeding in mineral springs in the Semliki forest in Uganda. *A. quadriannulatus*, found in south-east Africa and *A. quadriannulatus* B, which has been described in Ethiopia are not considered vectors of human malaria as they are generally zoophilic [3, 4].

A arabiensis is the principal malaria vector in Sudan. It is the most widespread member of the *A. gambiae* complex, dominant throughout most of the Afro-Tropical region, extending northwards along the River Nile to ≈ 20 ° N in Sudan. The only other vectors of any known importance in the Sudan are *A. nili* Theobald and *A. pharoensis* Theobald [7].

The disease transmission season may last from July/August to November/December, with earlier beginning of June in the southern areas and starts in August in northern areas. Urban Cities may have another transmission during winter season [5-10].

The objective of the present work is to identify the Anopheles species of this geographical region as part of the mosquito mapping program and identify their habitats. Such data will be of great value for the malaria Control Program and the decision –makers.

2. Materials and methods

2.1 Study Design

A cross-sectional design was followed [9].

2.2 Study Area

EnNahud Town is the largest Town in the west Kordofan State. It is geographically confined between latitudes 12, 68333 north and longitudes 28, 41667 east (map1). Its population is estimated at 144,702 inhabitants, unevenly distributed in 30 blocks. The Town lies in a savanna semi-arid zone (map2). The annual rainfall ranges between 200 – 700 mm. The wet season lasts for about four months from June to October. The monthly range of temperature during the summer season is about 30 – 40 °C from April to May, and the lowest temperature during the winter season is about 15 – 25 °C.

2.3. Sampling

2.3.1 Sample frame

2.3.2 Sampling units

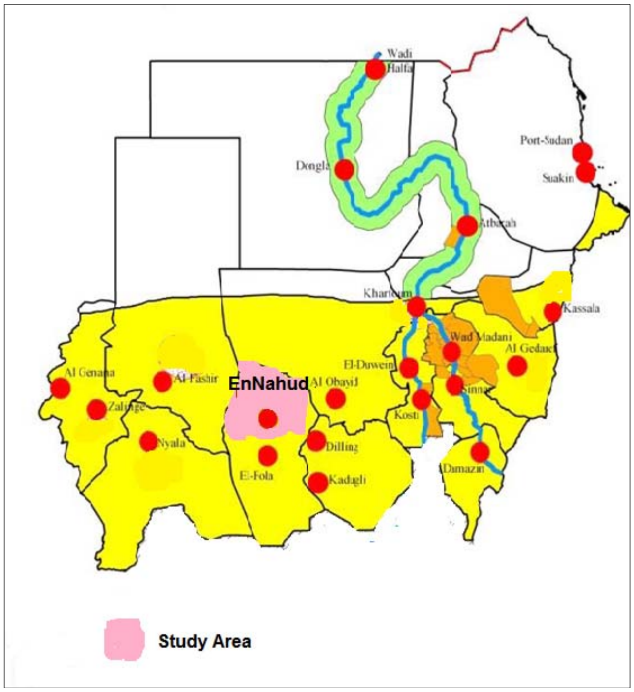
The primary sampling unit (PSU) is the sentinel site or station (all the breeding sites stagnant or temporary clean water), were found to be positive with *Anopheles* mosquitoes larvae, and adult *Anopheles* stages were found in houses in the targeted stations of EnNahud Town. The study was conducted in randomly selected using systematic random sampling, 8 stations (blocks; Algady, Alban Gadied, Um- Alqura, Abuglouve, Alnasr, Althawra, Aburnat, Abusnoon blocks) in study area for mapping of *Anopheles* spp mosquitoes in EnNahud Town - west Kordofan State.

The ultimate sampling unit is the positive breeding site of *Anopheles* species in each study station taken during the study period. Larvae of all available instars were collected from brick traditional factory (kaminas), freshwater, broken pipe, pools, water tanks, ponds (haffirs) used by animals for drinking, puddles and depressions made by human footprints and animal hooves; and other temporary or standing water. Sunlit temporary rain pools.

In addition to that, adults were collected from houses, four stations (blocks; Alban Gadied, Um-Alqura, Algady, Aburnat blocks) using spray sheet method [5], and selected systematic randomly.

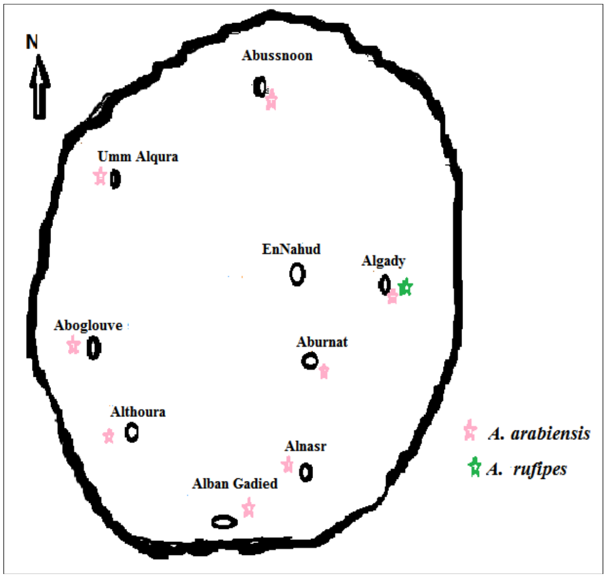
2.3.3 Sample size

Mosquito larvae of all stages were collected from 8 different breeding habitats in EnNahud Town.



Source: NMCP ,Sudan (2014).

Map 1: Map of Sudan



Map 2: Map of Study Area showing the location of the study block within EnNuhud town, West Kordofan State, Sudan

2.4 Data Collection and Processing
2.4.1 Larvae collection
The mosquito larvae were collected using a standard white ladle from large and small water bodies in the study stations (Algady, Alban Gadied, Um-Alqura, Aboglouve, Alnasr, Althawra, Aburnat, Abusnoon blocks), from July 2014- June 2015. Four trained malaria worker team under direct supervision from researcher had collected *Anopheles* mosquitoes, using dipping and netting techniques [5]. Larvae (2482) were transferred in vials to the laboratory, washed and killed with hot water and kept in 70% ethanol, then mounted on slides using DPX, and 30 larvae were reared in the insectary up to the adult stage.

2.4.2 Adult Collection
Adults (resting indoors) were collected from 4 stations (blocks), from July 2014 - June 2015. The stations were Alban Gadied, Um-Alqura, Algade, Aburnat blocks. Five rooms from each (block) were selected randomly taking into consideration the presence the breeding sites near the location. Before spraying, a permission was granted from the owners of the house; rooms were evacuated; the pyrethrum-spay catches method was adopted for adult collection. White sheets were sprayed over the entire floor of the room. The doors and windows were closed and the room was sprayed with 0.2% pyrethrum in kerosene. After 10-15 min, the dead mosquitoes were collected from the sheets in Petri dishes or bottles, transported to the laboratory for identification. The collection was carried out between 8:00 and 10:00 am.

2.5 Identification
The collected larvae and adults were identified by morphological characters, using the *Anopheles* mosquito keys of the Sudan for larvae and adults [11], and the key of Gillies and De Meillon [12]. All *A. gambiae* specimens were recorded as *A. arabiensis*, which is the only member of the complex found in eastern Sudan [13, 14].

2.5.1 Larvae
2.5.1.1 *A. gambiae complex (A. arabiensis)*

The 4th instar larvae were mounted [15] and classified using the keys of Gillies and De Meillon [12]. This species has simple inner clypeal hairs and widely separated; abdominal plate not more than 2/3 distance between palmate hair; both long mesopleural hair simple occasionally one split into 2-3 branches; inner shoulder hair poorly developed; basal tubercles very small or non- existent.

2.5.1.2 *A. rufipes*

The species is characterized by inner clypeal hairs widely separated; outer clypeal hairs simple or branched. Long hairs one feathered and the palmate hairs with short blunt- tipped filaments.

2.5.2 Adult
Adults were identified morphologically by light microscopy using the key of Gillies and De Meillon [12].

2.5.2.1 *A. gambiae complex (An. arabiensis):* Wing of adult female with more than one white spot on costa, abdominal segments 2-7 without laterally projecting tufts of hairs and the legs specked.

2.5.2.2 *A. rufipes:* Wings with more than one white spot on costa, abdominal segments 2-7 without laterally projecting tufts; legs not speckled; hind tarsus 4 and 5 both pale.

2.6 Data analysis
Data was analyzed using SPSS computer software program. Mean ± SE were calculated, in addition to descriptive statistics (percent and frequency).

3. Results
The study covered both the aquatic stages and the flying stage (adults).

3.1 Larval number and Density / habitat
A total of 2,482 anopheline mosquito larvae were collected from the 8 previously mentioned blocks during the sampling period specified in the previous section (12 months). Of these, 1287 (52.6%) were collected from pools, 736 (28.7%) from broken pipes, 180 (7.3%) from swamps, 279 (11.4%) from brick traditional factory (kaminas) as shown in table (1). Morphologically, 2,449 (98.7%) larvae were identified as *A. gambiae s*.l, and 33 (1.3%) as *A. rufipes*. The former species larvae were found in all investigated blocks. While *A. rufipes* specimens were found in Algady block only. The mean number of *A. gambiae s.l.* larvae and larval density according to different months are presented in table (1).

During July 2014, the larvae (means ±SE) were found in pools and swamp habitats (57.0 larvae), followed by pools + kaminas (35 larvae), while pools alone harbored 28.0 larvae, and 28.5 larvae for pools +broken pipes.The high density of *A. gambiae s.l.* Larvae was found in pools and bricks kaminas (3.0), followed by pools +swamp (2.0).

Pools + swamp registered during August high number of *A. gambiae s.l* larvae (89.0), followed by

Pools + bricks Kaminas (42.0), pools + broken pipes and pools alone registered (39.5).

The highest density of *A. gambiae s.l.* larvae was found in pools + swamp (3.0), followed by pools + kaminas (2.0). Pools alone (1.7) showed higher density than pools + broken pipes (1.2).

The highest total larvae was shown in pools + swamp (92.0), followed by pools alone (47.6), pools + broken pipes (45.0), and pools + kaminas (40.0). High density was shown in pool + swamps (3.0), followed by pools alone (2.8). The densities in the other two sites were not drastically different, *i.e*. 1.5 and 1.3 for the kaminas sites and the of broken pipes sites, respectively.

The highest during October was reported from pools +bricks kaminas (68.0).This was followed by pools + swamps (57.0). However, pools + broken pipes harbored 33 larvae, whereas pools alone harbored 49.6 larvae. Density ranged between 2.2 and 1.0. Pools alone showed the highest density; whereas the pools + broken pipes registered the lowest.

Table 1: Mean number of *A. gambiae s.l.* larvae and larval density during the sampling period (July 2014 to June 2015) from the different Blocks of EnNuhud Town, West Kordofan, Sudan.

Date	Habitat	No. of larvae (mean ± SE)	Density/dip
July 2014	Pools	28.0±6.2	1.6±0.4
	Pools + broken pipes	28.5±6.5	0.8±0.1
	Pools + bricks traditional factories (Kamina)	35.0±0.0	3.0±0.0
	Pools and swamp	57.0±0.0	2.0±0.0
August 2014	Pools	32.5±5.4	1.7±0.3
	Pools + broken pipes	39.5±2.5	1.2±0.2
	Pools +bricks traditional factories (Kaminas)	42.0±0.0	2.0±0.0
	Pools +swamp	89.0±0.0	3.0±0.0
September 2014	Pools	47.6±2.6	2.8±0.3
	Pools +broken pipes	45.0±0.0	1.5±0.0
	Pools +bricks traditional factories (Kamina)	40.0±0.0	1.3±0.0
	Pools +swamp	92.0±0.0	3.0±0.0
October 2014	Pools	49.6±7.2	2.6±0.3
	Pools + broken pipes	33.0±0.0	1.0±0.0
	Pools + bricks factories (Kamina)	68.0±0.0	2.2±0.0
	Pools +swamp	57.0±0.0	2.0±0.0
November 2014	Pools	50.5±5.9	2.7±0.5
	Pools + broken pipes	34.0±*0.0*	1.0±*0.0*
	Pools +bricks factories(Kamina)	72.0±*0.0*	2.4±*0.0*
	Pools +swamp	67.0±*0.0*	2.0±*0.0*
December 2014	Pools	16.2±6.8	1.3± 0.5
	Bricks factories (Kamina)	51.0±0.0	5.6±0.0
	Pools +broken pipes	23.0±0.0	0.8±0.0
	Pools +swamp	0.0±0.0	0.0±0.0
January 2015	Pools	5.8±3.5	0.5±0.3
	Kaminas	28.0±0.0	3.0±0.0
	Pools +broken pipes	18.0±0.0	.6±0.0
February 2015	Pools	5.8±3.9	0.4± 0.2
	Kaminas	19.0±0.0	1.4±0.0
	Pools + broken pipes	22.0±0.0	0.7±0.0
March 2015	Pools	3.8±2.4	0.2± 0.1
	Kaminas	20.0±0.0	1.3±0.0
	Pools + broken pipes	8.0±0.0	0.3±0.0
April 2015	Pools	4.0±2.5	0.2±0.1
	Kaminas	12.0±0.0	0.8±0.0
	Pools + broken pipes	12.0±0.0	0.4±0.0
May 2015	Pools	6.7±5.4	0.5± 0.3
	Kaminas	13.0±0.0	0.9±0.0
	Pools + broken pipes	38.0±0.0	1.2±0.0
June 2015	Pools	8.1±10.5	0.3±0.5
	Pools + broken pipes	35.0±0.0	1.1±0.0
	Pools + Kaminas	61.0±0.0	2.0±0.0

The total during November was found in pools + bricks kaminas habitats (72.0), followed by pools+ swamp (67.0). The high larval density was found in pools alone (2.7), followed by pools +bricks kaminas (2.4).

For December (winter), bricks kaminas habitats harbored (51.0), followed by pools+ broken pipes (23.0), while pools alone (16.2 larvae) and none was detected in pools +swamp habitat. The high density of was found in kaminas (5.6), followed by pools alone (1.3).

During January 2015, the pools around swamps and kaminas disappeared. The high number was shown in kaminas (28.0), followed by pools + broken pipes (18.0), and pools (5.8 larvae). High density/site during this month registered in kaminas (3 0).

During February, pools + broken pipes (22.0), kaminas (19.0), and the lowest was for pools alone (5.8 larvae). As expected, density/site was higher in kaminas (1.4). However, during March (spring), the number of. larvae/ site was lower than that of January, showing the highest in kaminas habitats (20.0) , followed by pools + broken pipes (8.0), while pools alone recorded (3.8). During April (Summer), the highest value was for both the pools +broken pipes and kaminas (12.0), and pools harbored almost 1/3rd of the former two habitats population (*i.e*. 4.0 larvae). High density/ site was shown in kaminas habitat (0.8).

During May, pools + broken pipes habitats registered (38.0 larvae), followed by pools + Kaminas habitats (13.0). The high density/ site was found in pools + broken pipes (1.2). And Kaminas (0.9). The highest number was reported during June, just before the rainy-season was from pools+ kaminas (61.0 larvae), which was the highest in this study throughout the year, followed by pools+ broken pipes (35.0), and the least number/site was found in pools alone (8.1). Density/site during June ranged between 2.0 and 0.3.

3.2 Larval number and density / Block

Um-Alqura block of the town showed high number of *A. gambiae s.l.* larvae in pools + swamps (60.3 larvae), followed by pools (3.2). Pools + swamp habitat in this area showed the highest density (2.0) in all stations. However, the pool habitats in the area ranked VI among all stations densities (0.1; Table 2).

For Abuglouve block (Table 2**)**, two habitats were found, i.e. pools, and pools+ broken pipes.

The highest number was number of larvae was detected in the pools + broken pipes (38.0) habitat; ranked II among all sites of the town, followed by pools (13.3 larvae); ranked VI. The highest density of larvae/site was reported was (1.3) in the pools habitat, followed by pools +broken pipes (1.2).

The difference was not statistically significant.

Althawra block was characterized by the presence of pools only. The larval number was 16.8, and ranked fifth among the sites in the town. Larval density (1.1) in the pools is ranked VII.

Regarding Aburanat block, also only pools were present as habitat for the larvae. The mean was 30.2, which is ranked as the third among all sites in town. larval density was 1.3, ranking as the third also among the others.

The sixth block was Abusnoon block. It contained pools only. The mean was 26.5 larvae.

It ranked fourth. Larval density in this block was 1.5; ranked second.

Table (3) shows that the prevalence and distribution of *A. gambiae s*.l. in different blocks of the town and the habitats. The table also showed that the major breeding site of this species was in pools (52.6%), followed by broken pipes (28.7%), bricks Kaminas (11.4%) and swamps (7.3%).

A. rufipes (33 larvae only) breeding was restricted to the broken pipes pools (100%).

Table 2: Mean of *A. gambiae s.l.* larvae and larval density in different blocks of EnNhud Town, West Kordofan State, Sudan

Block	Habitat	No. of Larvae /site & rank	Density (no. of larvae/ no of scoops) & rank
Um-Alqura	Pools	3.2±3.2 VIII	0.1±0.1 VI
	Pools +swamp	60.3±0.0 I	2.0±0.0 I
Abuglouve	Pools	13.3±2.2 VI	1.3±.4 IV
	Pools +broken pipes	38.0±1.9 II	1.2±0.1 V
Alnasr	Pools	13.1±5.4 VII	0.8±0.3 VIII
Althawra	Pools	16.8±6.3 V	1.1±0.4 VII
Aburnat	Pools	30.2±4.1 III	1.3±0.1 III
Abusnoon	Pools	26.5±8.7 IV	1.5±0.5 II

Table 3: Distribution of *Anopheline* species in different breeding habitats within Enuhud town Blocks, West Kordofan State, Sudan

Type of breeding site	Total no. larvae collected & rank	Species identification			
		A. gambiae s.l		*A. rufipes*	
		No.	%	No.	%
Pools	1,287 I	1,287	52.6	0	0.0
broken pipes	736 II	703	28.7	33	100
Swamp	180 IV	180	7.3	0	0.0
Bricks kaminas	279 III	279	11.4	0	0.0
Total	2482	2,449	100.0	33	100

% *A. gambiae* = 98.7%, % *A. rufipes* = 1.3%

4. Discussion

A. arabiensis is the predominant member of *A. gambiae s.l.* in northern Sudan. It is the principal malaria vector in the Sudan [13, 14, 16]. This species is the most widespread member of the *A. gambiae* complex, abundant throughout most of the Afro-Tropical region, extending northwards along the River Nile to ≈ 20 ° N in Sudan [17]. These findings agree with the present work results which, indicated that *A. arabiensis* was widely present throughout the year, during the rainy-season and the dry-season, summer, autumn and winter in the study area, *i.e.* EnNohud town, west Kordofan state. The larvae were detected wherever water is available in the form of pools, swamps, broken pipes, etc.

The immature stages were collected from all designated blocks of EnNohud town. This might be attributed to the availability and suitability of the investigated habitats conditions, *e.g.* broken pipes and bricks Kaminas, in addition to water pools exposed to sunlight, which is preferred by *A. arabiensis*.

The highest density of *A. arabiensis* was recorded in water pools during the period from June to November (rainy-season). These pools are existing in all blocks of EnNohud town, since the drainage system is not properly functioning or non-existing. By the progress of the rainy-season and the increasing rains, breeding sites can be in the form of swamps and pools, depending on the terrain (ups and downs), and some anthropogenic activities. One of the major anthropogenic activities that has an impact on the presence of mosquitoes throughout the year in the town is the traditional bricks factories (kaminas) during the period from December to April (dry-season), including the winter. Therefore, there is an urgent need to conduct routine larval control in kaminas area as a source of the vector, in addition to conducting some studies in the kaminas areas aiming at reducing the availability of exposed water as a breeding site for mosquitoes.

Regarding the swamps, which recorded highest larval density during August, fortunately, they disappeared (dried) from January to June 2015. Some engineering efforts must be done to prevent the formation of such swamps. The broken pipes record highest larval detected during May, *i.e.* before the start of the rains. Thus, it can be said that it is one of the major reasons and sources for the continued presence of mosquitoes in the town. For the rest of the year, pools formed by broken pipes, continue to maintain what can be considered as a suitable medium for breeding and as foci for the next generations.

All these conditions are available in the study area. This study confirmed that *A. arabiensis* can survive even in arid areas and also during the dry-seasons of the year.

In spite of data recorded that the other Anopheline mosquitoes present in Sudan, such as *A. nili*, *A. dthali* and *A. rufipes* are of no medical importance [13, 16], The intensity of population of each species differs; one species may be extensively numerous and abundant and the other may be represented by a single larva [18]. This study findings emphasized the presence of *A. rufipes* (1.3%), while *A. arabiensis* (98.7%) in El Nahud Town.

5. Conclusion and Recommendation

5.1 Conclusion

1. *A. arabiensis* is widely distributed in EnNohud town.
2. The results of this study confirmed that high density of Anopheles in pools breeding habitat during rainy-season from June to November, while high density of Anopheles recorded in bricks factories (kaminas) during December to April (dry- season).
3. These pools are existing in all blocks of EnNohud town, since the drainage system is not properly functioning or non-existing.
4. One of the major anthropogenic activities that has an impact on the presence of mosquitoes throughout the year in the town is the traditional bricks factories (kaminas) during the period from December to April (dry-season),

5.2 Recommendations

1. More studies are needed in order to assess the distribution of all and new species in the different seasons in addition to its role in malaria transmission.
2. The environment must be kept clean and clear of stagnant water
3. Malaria vector mapping and distribution must be continuously monitored, at least on yearly basis, for its importance for decision-making to assist in applying appropriate control measures.
4. It is also important to monitor for the status of each species and its population incidence so as to safeguard against invasive species.
5. Molecular techniques must by adopted for verification of all species
6. Environment management (preventive actions), manipulation and modification, especially the drainage system, the Kaminas activities, and drinking water pipes network maintenance must be under close observations, maintenance and emergency funds must be available for corrective actions.
7. Indoor Residual spraying must be intensified during the month of November, December and January every year.
8. Similar studies must be conducted in the rest of the state Towns and villages so as to complete the state map.

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