# ARGAS (PERSICARGAS) PERSICUS LIFE CYCLE UNDER CONTROLLED AND OUTDOOR CONDITIONS \*

BY

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#### ABSTRACT

Argas (Persicargas) persicus (Oken, 1818) is the type species of the subgenus and is the vector of several agents infectious to humans and to birds. Comparative data on its life cycle under laboratory conditions (30-32°C, 75 % RH, pigeon hosts) and under Cairo area natural outdoor conditions are presented. The cycle requires 63-178 days in the laboratory, 111-260 days outdoors. Except for larvae and third-instar nymphs (N<sub>3</sub>), the survival period of starved ticks is longer in the laboratory (L, N<sub>1</sub>, N<sub>2</sub>, N<sub>3</sub>,  $\circlearrowleft$ ,  $\circlearrowleft$ : 34, 108, 77, 199, 292, 293 days, respectively) than outdoors (56, 34, 52, 248, 154, 161 days, respectively). Copulation is essential to obtain viable eggs. Maturation age for the female is 7 days, for the male is 2 days. Feeding is not essential for male viability. During winter-early spring (November-April) outdoors, females undergo ovarian diapause and few feed; in the laboratory feeding and oviposit continue throughout the year except in February.

Female fecundity (in the laboratory) is reduced after repeated feedings Under the conditions cited,

parent adults must be replaced from nature to maintain a strong laboratory colony.

## RÉSUMÉ

Argas (Persicargas) persicus (Oken) 1818 est l'espèce typique du sous genre et le vecteur de plusieurs agents infectieux à l'homme et aux oiseaux. Le cycle biologique dure de 63 à 178 jours au laboratoire, de 111 à 260 jours dans les conditions naturelles. A l'exception des larves et des nymphes au  $3^{\rm e}$  stade (N<sub>3</sub>) la survie des tiques à jeun est plus longue dans les conditions du laboratoire. Pour les L, N<sub>1</sub>, N<sub>2</sub>, 33, 99 elle est de : 34, 108, 77, 199, 292, 293 jours respectivement alors que dans les conditions naturelles elle est de : 56, 34, 52, 248, 154, 161 jours respectivement. La copulation est essentielle pour obtenir des œufs viables. L'âge de la maturité des femelles est de 7 jours tandis que celui du mâle est de 2 jours. La prise de sang n'est pas essentielle pour les mâles. Durant l'hiver (novembre-avril), dans les conditions naturelles, les femelles refusent de se nourrir et entrent en état d'hibernation; un petit nombre accepte de manger. Ceci ne se remarque pas au laboratoire à l'exception du mois de février. La répétition de prise de sang par les femelles au laboratoire diminue leur fécondité. Dans ces conditions, les parents adultes devraient être remplacés pour maintenir une forte colonie au laboratoire.

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#### Introduction

The life cycle, biological properties, and medical and veterinary importance of tick populations attributed to Argas (Persicargas) persicus (Oken, 1818) have been reported in numerous publications from North and South America, Europe, Asia, Africa, and Australia (Hoogstraal, 1970). However, continuing collaborative research by the NAMRU-3 Medical Zoology Department and the Rocky Mountain Laboratory has shown that many of these reports were based on other species misidentified as A. persicus (some examples were cited by DIAB & SOLIMAN, 1977). This study of the A. (P.) persicus life cycle under controlled laboratory and outdoor field conditions in Egypt was undertaken to determine biological parameters for planning biomedical and virological research on this species and compare the biological dynamics of this related Argas species.

## METHODS AND MATERIALS

Argas (P.) persicus was collected from chicken houses, Maadi area, Cairo, October 1975. Rearing methods are adapted from those applied in the NAMRU-3 Medical Zoology laboratories (Kaiser, 1966). Ticks were reared in an insectary regulated to 30-32°C and 75 % RH to study the life cycle under laboratory conditions, or held outdoors subject to natural weather and photoperiod conditions. Hosts were domestic pigeons which were used repeatedly for adults and nymphs, and once or twice for larvae. Parasitic and incubation periods and longevity were observed under controlled and outdoor conditions, but readiness to attack parthenogenesis, and maturity age were studied only under controlled conditions.

# Immature stages

Readiness to feed. — Pools of  $\pm$  300 larvae 1-20 days after hatching were fed on pigeons; fully engorged larvae were counted daily until the last dropped. Batches of 50 N<sub>1</sub>, N<sub>2</sub>, N<sub>3</sub>, one day after molting were placed to feed on pigeons. Those that did not feed within 2 hours were placed to feed the following day until all had fed. Numbers of fed and unfed nymphs were counted daily.

Longevity. — Batches of  $\pm$  200 unfed larvae and of 50-100 N<sub>1</sub>, N<sub>2</sub>, N<sub>3</sub> were held immediately after molting during June to avoid possibility of diapause; the number of dead ticks were observed and recorded every other day.

## Adult stage

Fecundity. — One hundred pairs of fed females and males, each pair in a tube, were observed daily during and following 3 feeding periods for oviposition, egg incubation period, percentage of hatching, and prehatching period. A batch of 120 pairs of adults was fed 10 times and observed for effect of age and repeated feeding on fecundity.

Parthenogenesis. — A batch of 80 virgin females was prepared by isolating  $N_3$  in separate tubes until molting. Virgin females were fed 3 times at 1-month intervals and checked daily for egg laying.

Readiness to feed. — Adults were placed on pigeons to feed 1-18 days after molting.

Longevity. — Adults which had previously fed were held without further food and observed every other day until all died.

Maturation age of males and females. —  $300 N_3$  were held individually in separate tubes and observed daily for molting. Emmerged adults were fed and paired, each in a separate tube, as follows:

- a) 1-7 day old males paired with 7-8-day old females.
- b) 1-7 day old females paired with 7-8-day old males.
- c) Pairing between sexes of equal age (9-18-day old).

Males were left for 24 hours with females and then removed; afterward females were checked daily for oviposition and egg viability.

The mean and standard deviation (SD) were calculated.

#### RESULTS

# Egg stage. —

Newly laid eggs are spherical, shiny, pale brown; after 2 days they become dark brown. Two days before hatching, eggs are dry and scattered around the tube, half of each egg is white and the embryo is visible through the shell. Incubation required 4-22 (mean 12.1) days in the laboratory and 4-91 (mean 20.0) days outdoors, Table 1.

## Larval stage. —

Few 1-3 day old larvae attached; 74 % and 93 % attached when 4 and 6 days old, respectively, 73-89 % when 7-15 days old, and 30-37 % when 16-20 days old. Larval feeding and premolting periods (days), respectively, were 4-16 (mean 7.3) and 2-17 (mean 7.1) in the laboratory and 7-13 (mean 9.7) and 7-19 (mean 13.5) outdoords (Table 1). In both environments, 9-10 % of the premolting larvae died and 12-15 % died while molting or never molted. Unfed larvae survived 32-37 (mean 34.2) days in the laboratory and 49-65 (mean 56.0) days outdoors (Table 1, Fig. 1).

## Nymphal stage. —

First instar  $(N_1)$ . — In the laboratory, ca. 16 % of the  $N_1$  fed on day 1 postmolting, 50 % fed on day 2, all fed by day 14. Outdoors, these percentages were 6, 70, and 100, respectively. Feeding was completed 20-40 min after placement on hosts. (In winter, nymphs of each instar wandered over the host for several hours before feeding.) Premolting and longevity periods are shown in Table 1 and Fig. 2.

Second instar  $(N_2)$ . — On day 4 postmolting, 32 % of the laboratory reared  $N_2$  fed and 88 % of the outdoor  $N_2$  fed. On day 8 in both environments, all  $N_2$  fed. Feeding was completed 20-30 min after placement on the hosts. Premolting and longevity periods are shown in Table 1 and Fig. 3.

Third instar  $(N_3)$ . — On days 2 and 8 postmolting, 50 % and 100 %, respectively, of  $N_3$  from both environments fed. The nymphal to adult premolt period was 10-22 (mean 20.6) days for outdoor  $N_3$  and 6-14 (mean 9.2) days for laboratory  $N_3$ . Most  $N_3$  from both environments held for longevity tests survived for 278 (mean 248) (outdoors) and 215 (mean 198) (laboratory) days, Table 1. Of laboratory reared  $N_3$ ; 7.1 % (6/84) molted to  $N_4$ ; 28.6 % (24/84) to males; and 64.3 % (54/84) molted to females.

Table I. — Life cycle of Argas (Persicargas) persicus reared outdoor and under regulated (30-32°C, 75 % R.H.) conditions.

			Perio	ys				
Developi	nental -	Lab	oratory		Outdoor*			
stage		Mean + SD**	Range	Mode	Mean + SD**	Range	Mode	
Egg prehatching		12.1 <u>+</u> 1.7	4-22	13	20.0 <u>+</u> 15.0	7-91	13	
Larva:	feeding	7.3 <u>+</u> 1.2	4-16	6	9.7 <u>+</u> 3.1	7-13	7	
	premolting	7.1 <u>+</u> 1.5	2-17	4	13.5 <u>+</u> 1.0	7-19	13	
	longevity	34.2 ± 0.3	32-37	34	56.0 ± 0.8	49-65	49	
Nymph <sub>I</sub>								
	premolting	9.0 + 1.2	5-17	10	18.5 ± 0.6	15-22	15	
	longevity	108.7 ± 22.5	18-170	129	34.4 <u>+</u> 8.6	12-71	41	
$_2$ Nymph	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,							
	premolting	10.5 ± 1.2	5-15	19	25.1 <u>+</u> 0.6	12-36	14	
	longevity	77.5 <u>+</u> 24.0	22-133	74	52.7 <u>+</u> 11.5	28-27	52	
Nymph <sub>3</sub>			a de parte parte de la como					
	premolting	9.2 ± 0.6	6-14	9	20.6 <u>+</u> 2.8	10-22	14	
	longevity	198.7 <u>+</u> 22.4	109-218	215	248.5 <u>+</u> 48.8	74-297	278	
9 prefeeding		1	-	_	-	_	-	
preoviposition		7.9 ± 2.1	3-27	7	10.7 <u>+</u> 3.0	3-31	9	
no.	eggs/	23.8 ± 10.3	5-70	50	25.1 <u>+</u> 6	5-60	30	
long	evity	293.4 ± 9.3	265-308	300	161.6 <u>+</u> 30.4	95-223	210	
o prefee	eding	2	-	-	-	_	-	
long	evity	292.5 <u>+</u> 10.4	265-308	265	154.2 <u>+</u> 32	95-223	157	
Sex ratio	o: =	1.0	00:2.25		The state of the s			

<sup>\*</sup> No egg laying, or larvae observed between November and April. \*\* Standard deviation.

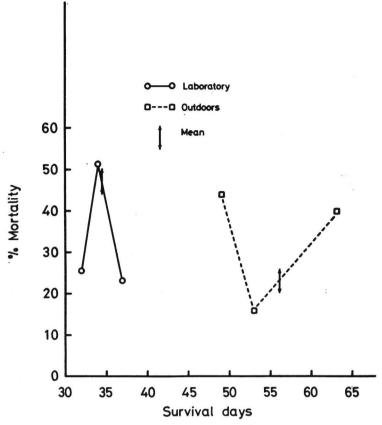


Fig.1, Longevity of unfed <u>Argas (P.) persicus</u> larvae held outdoors and in the insectary.

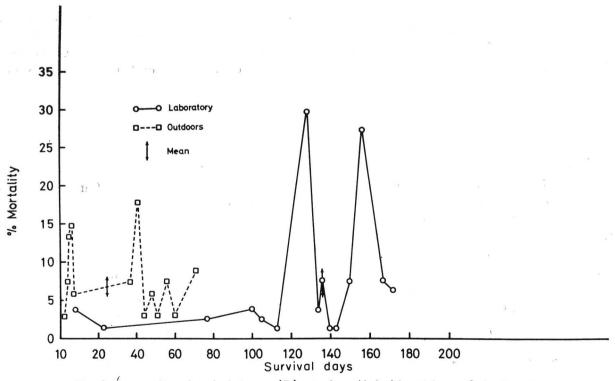


Fig. 2 , Longevity of unfed Argas (P.) persicus  $N_1$  held outdoors & in the insectary .

## Adult stage. —

The percentages (approximate) of adults feeding outdoors during winter-early spring were: December (39  $\mathbb{Q}$ , 34  $\mathbb{Z}$ ), January (66  $\mathbb{Q}$ , 46  $\mathbb{Z}$ ), February (0), March (0), April (81  $\mathbb{Q}$ , 79  $\mathbb{Z}$ ) (Fig. 4). There was no oviposition between November and the end of April.

The percentages of adults feeding in the laboratory at the same time were: December (86  $\circlearrowleft$ , 80  $\circlearrowleft$ ), January (77  $\circlearrowleft$ , 60  $\circlearrowleft$ ), February (72  $\circlearrowleft$ , 81  $\circlearrowleft$ ) (Fig. 4). In March and afterward, most (ca. 90 %) adults fed. In February, there was no oviposition (Fig. 5).

Effect of age and repeated feeding on fecundity. — The percentage of ovipositing females decreased from 85.2 to 20 % after 10 meals (Table 2). Egg viability also decreased after 9 feeds from lay 81.6 to 33.3 % (percentage of hatched eggs). None of the 3 females surviving to the 11th feed could lay eggs. Preoviposition and prehatching periods were unaffected by repeated feeding (Table 2). In all conditions, most females oviposited in one batch, very few laid 2-5 eggs first, then 2-3 days later laid the rest of the eggs.

Parthenogenesis. — No parthenogenetic development was observed after repreated feeding in 80 virgin females. No fertile or unfertile eggs were laid. These females fed for the normal period (30-40 min).

Maturation age. — Females less than 7 days old did not oviposit. Females 7-8 days old mated with 1-day old males also did not oviposit but those mated with 2-7-day-old males did oviposit even when the males were unfed. Matings between adults of the same age (9-18-day-old) laid viable eggs. The preoviposition period was shorter (8-11 days) when females were older than 8 days than in those that were 7 days old (14-26 days). Adults held outdoors could not be compared because of the winter conditions prevented egg laying.

Readiness to feed. — From 50-60 % of the females held outdoors or in the insectary fed on the day of molting to 5 days old, and most fed on day 6. Males less than 2 days old did not feed but fed when 3 days old.

Longevity. — Maximum survival times are 308 (mean 3292, 9293) days under controlled conditions, and 223 (mean 3154, 9161) days outdoors. One female lived for about 876 days (collected from the field on 19 October 1975, died 14 March 1978) and had no food for 260 days (after 27 June 1977). One male survived for 624 days (collected 19 October 1975, died 6 July 1977).

## DISCUSSION

The Argas (P.) persicus life cycle from egg to adult under controlled conditions (30-32°C and 75 % R.H.) requires 2.5 to 3 months during spring and summer and 4.5 to 6 months during fall and winter. This difference is due to diapause or slower rates of adult feeding, egg laying and larval feeding, and to the prolonged developmental periods of all stages. Under outdoor conditions, the life cycle is more affected by winter; no females fed during February and March, even when hosts were introduced and no egg laying was observed from November to April (Fig. 4). Under laboratory conditions, females were able to break diapause by feeding and ovipositing during winter. The developmental pattern of A. (P.) persicus is close to that of A. (A.) hermanni (Khalil and Metwally, 1974), but differs from that observed in the related species, A. (P.) arboreus, which diapause in winter even under controlled conditions (Kaiser, 1965; Guirgis, 1971; Khalil, 1974; K. M. El Kammah, unpub.). The ability of A. (P.) persicus to feed in winter and the abundance of its main host, chickens, all the year increase the role of this species

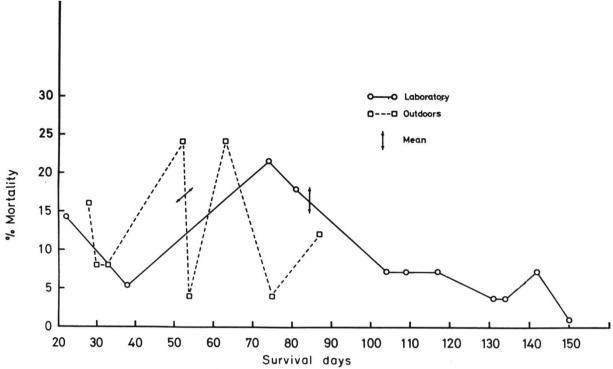


Fig. 3 , Longevity of unfed Argas (P.) persicus  $N_2$  held outdoors & in the insectary .

Table II. — Effect of repeated feeding on oviposition and hatching of laboratory-reared Argas (P.) persicus under regulated (30-32°C, 75 % R.H.) conditions.

					No. days before			
Meal	Ovipositing 9		Hatching		Ovipositing 9		Hatching	
no.	No.	%	No. batche	es %	Mean	Range	Mean	Range
1	98/115	85.19	80/98	81.63	8.07	3-27	12.0	5-20
2	63/99	63.64	43/63	68.25	7.11	3-19	10.89	5-16
3	55/106	51.89	34/54	62.96	7.64	3-15	12.44	4-31
4	45/86	52.33	23/45	51.1	8.19	4-16	15.48	5-22
5	26/64	40.63	12/26	46.15	7.69	3-14	12.5	7-20
6	6/23	26.09	4/6	66.67	6.67	6-9	11.25	4-45
7	5/21	23.81	3/5	60.0	7.0	5-10	14.67	8-18
8	4/22	18.18	2/4	50.0	7.5	5-13	11.5	8-15
9	3/15	20.0	1/3	33.33	10.33	8-15	-	15
10	1/5	20.0	0		7	7	-	-
11	0/3	-	-	-	-	-	-	-
12	0/2	_	-	-	-	-	-	-

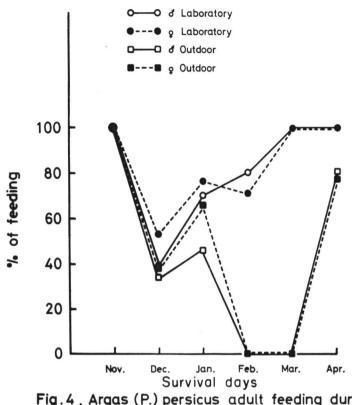
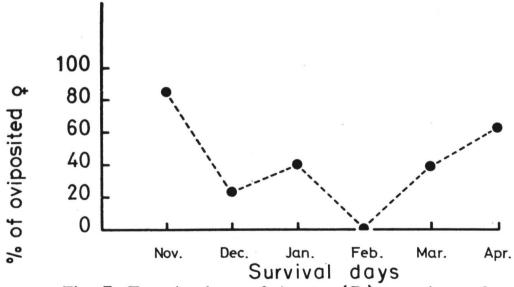


Fig. 4, Argas (P.) persicus adult feeding during winter under insectary or outdoor conditions.



Survival days
Fig. 5, Egg laying of <u>Argas</u> (P.) <u>persicus</u> females
during winter under controlled conditions.

in maintaining or transmitting rickettsia and spirochetes to poultry (Neitz, 1956; Roshdy, 1961; Diab and Soliman, 1977).

Copulation is essential for egg laying, no parthenogenetic development was observed as in A. (P.) arboreus (Khalil, 1969), but Zakia et al. (1978) suspect the presence of parthenogenesis in A. (P.) arboreus. As observed by Balashov (1968) and Tatchell (1962) feeding is not essential for A. (P.) persicus male to copulate. For A. (P.) persicus females, bloodmeal is essential for oviposition. Maturation of female reproductive system is not completed before 7-8 days old even if they are fed. Shanbaky and Khalil, 1975, also found that the bloodmeal is essential for A. (P.) arboreus females to complete oogenesis.

A. (P.) persicus females in this study oviposited fewer eggs than A. (P.) arboreus (HAFEZ et al., 1972), probably owing to greater amount of ingested blood by arboreus (TATCHELL, KERR and BOCTOR, 1973).

No larvae are available in winter in the field as a result of the lack of oviposited females. They are very few under regulated conditions for the same reason. Nymphs survive winter time and, if fed, molt at normal periods. In summer developmental periods of immatures is prolonged and longevity is shorter outdoors than in the insectary, probably because of the fluctuating temperature between day and night outdoors. The capability of this species to survive winter, even if the host is not available, indicates its economic importance as a widely distributed parasite of domestic chickens.

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## REFERENCES

- Balashov (Yu. S.), 1968. Bloodsucking ticks (Ixodoidea) vectors of diseases of man and animals. Akad. Nauk SSSR, Zool. Inst., Leningrad. 319 p. (1967). (In Russian) (in English: Misc. Publ. Ent. Soc. Amer. 8: 161-376).
- DIAB (F. M.) & SOLIMAN (Z. R.), 1977. An experimental study of *Borrelia anserina* in four species of *Argas* ticks. Z. Parasitenk, **53**: 201-212.
- Guirgis (S. S.), 1971. The subgenus *Persicargas* (Ixodoidea, Argasidae, *Argas*). 11. Ecology and seasonal dynamics of A. (P.) arboreus Kaiser, Hoogstraal & Kohls in Egypt. J. Med. Ent. 8: 407-414.
- HAFEZ (M.), ABDEL-MALEK (A. A.) & GUIRGIS (S. S.), 1971. The subgenus *Persicargas* (Ixodoidea, Argasidae, Argas). 12. Biological studies on the immature stages of A. (P.) arboreus Kaiser, Hoogstraal and Kohls in Egypt. J. Med. Ent. 8: 421-429.
- HAFEZ (M.), ABDEL-MALEK (A. A.) & GUIRGIS (S. S.), 1972. The subgenus *Persicargas* (Ixodoidea, Argasidae, Argas). 14. Biological studies on the adult stage of A. (P.) arboreus Kaiser, Hoogstraal and Kohls in Egypt. J. Med. Ent. 9: 19-29.
- HOOGSTRAAL (H.), 1970. Bibliography of ticks and tickborne diseases. from Homer (about 800 B.C.) to 31 December 1969. Vol. 2. Authors F-M. NAMRU-3, Cairo. 495 p.
- HOOGSTRAAL (H.), GUIRGIS (S. S.), KHALIL (G. M.) & KAISER (M. N.), 1975. The subgenus *Persicargas* (Ixodoidea: Argasidae: *Argas*). 27. The life cycle of *A.* (*P.*) *robertsi* population samples from Taiwan, Thailand, Indonesia, Australia, and Sri Lanka. South Asian J. Trop. Med. Pub. Hlth. 6: 532-539.

- KAISER (M. N.), 1966. The subgenus *Persicargas* (Ixodoidea, Argasidae, *Argas*). 3. The life cycle of A. (P.) arboreus and a standardized rearing method for argasid ticks. Ann. Entomol. Soc. Amer. 59: 496-502.
- KAISER (M. N.), HOOGSTRAAL (H.) & KOHLS (G. M.), 1964. The subgenus *Persicargas*, new subgenus (Ixodoidea, Argasidae, *Argas*). 1. A. (P.) arboreus, new species, an Egyptian *Persicus*-like parasite of wild birds, with a redefinition of the subgenus *Argas*. Ann. Entomol. Soc. Amer. 57: 60-69.
- KHALIL (G. M.), 1969. Biochemical and physiological studies of certain ticks (Ixodoidea). Gonad development and gametogenesis in *Argas* (*Persicargas*) arboreus Kaiser, Hoogstraal, and Kohls (Argasidae). J. Parasit., 6: 1278-1297.
- KHALIL (G. M.), 1974. The subgenus *Persicargas* (Ixodoidea: Argasidae: Argas). 19. Preliminary studies on diapause in A. (P.) arboreus Kaiser, Hoogstraal & Kohls. J. Med. Ent. 11: 363-366.
- KHALIL (G. M.) & METWALLY (S. A.), 1974. Observations on the subgenus Argas (Ixodoidea: Argasidae, Argas). 8. The life cycle of A. (A.) hermanni. J. Med. Ent. 11: 355-362.
- Neitz (W. O.), 1956. Classification, transmission, and biology of piroplasmas of domestic animals. Ann. N. Y. Acad. Sci., 64: 56-111.
- ROSHDY (M. A.), 1961. Observations by electron microscopy and other methods on the intracellular Ricktettsia-like microorganisms in *Argas persicus* Oken (Ixodoidea, Argasidae). J. Insect Pathol., 3: 148-166.
- TATCHELL (R. J.), 1962. Studies on the male accessory reproductive glands and the spermatophore of the tick, *Argas persicus* Oken. Parasit., **52**: 133-142.
- TATCHELL (R. J.), KERR (J. D.) & BOCTOR (F. N.), 1973. Biochemical and physiological studies of certain ticks (Ixodoidea). Haemolysis rate and meal size in the interactions between Argas (Persicargas) arboreus Kaiser, Hoogstraal and Kohls, A. (P.) persicus (Oken) (Argasidae) and some avian hosts. Parasit., 67: 41-51.
- ZAKIA M. RIAD, SIDKY (H. S. A.) & ABDEL-WAHAB (K. S. E.), 1978. Chromosomal pattern of Argas (Persicargas) arboreus. J. Egypt. Soc. Parasit., 8: 9-15.

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