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SCANNING ELECTRON MICROSCOPY
AND COMPARATIVE MORPHOLOGY OF ARGASID LARVAE
(ACARI: IXODIDA: ARGASIDAE)
INFESTING BIRDS IN EGYPT.

BY SOBHY ABDEL-SHAFY

(Accepted December 2004)

ARGAS ARBOREUS,
A. HERMANNI, A. PERSICUS,
CHAETOTAXY, LARVA,
SCANNING ELECTRON MICROSCOPY, MORPHOLOGY.

SUMMARY: The differentiation between Argas larvae; A. persicus, A. arboreus and A. hermanni infesting birds in Egypt was studied using Scanning Electron Microscopy and morphometric analysis. Results show that larval A. persicus, A. arboreus and A. hermanni carry 26, 27 or 28, and 23 pairs of dorsal setae, respectively. A. arboreus differs from A. persicus by the presence of two pairs of additional median setae. A. hermanni differs by the presence of an additional pair of dorsolateral setae and absent of anterosubmedian setae. Body setae of A. arboreus and A. hermanni longer than in A. persicus. Length and width of body were equal in A. persicus and A. arboreus but smaller in A. hermanni. Dorsal plate displayed very distinct bulging cells in A. hermanni, shallow cells in A. arboreus and slightly bulging wrinkles in A. persicus. This plate is longest in A. hermanni than others. Dental formulae of hypostome were 3/3, 3/3, and 4/4 in A. persicus, A. arboreus and A. hermanni, respectively. The numbers of denticles were 7/6/2, 8/8/3 and 10/9/3/2 in A. persicus, A. arboreus and A. hermanni, respectively. The corona of hypostome was bluntly rounded in A. persicus, slightly rounded in A. arboreus and distinctly rounded in A. hermanni. Hypostomal length of A. hermanni and A. arboreus were equal and larger than that of A. persicus. Palps were the longest in A. arboreus followed by A. persicus and A. hermanni. Chaetotaxy of palps was similar in all three species. Distances between posthypostomal setae (Ph1 and Ph2) were longer in A. persicus and A. arboreus than in A. hermanni.


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Acarologia, 2005. XLV, 1 : 3-12.
Introductions

The tick genus *Argas* plays an important role in the transmission of disease agents, which cause economic losses such as reduction in egg production or death of birds. Vermeil et al. (1996) showed that 12 species of *Argas* are known to carry about 20 viruses. Madbouly et al. (1990) reported that transovarial and transstadial transmission of *Borrelia anserina* (avian borreliosis) occurred in *Argas persicus*. Abdel-Shafy, (2000) revealed that an infected *A. persicus* could transmit the spirochetes to chicken more than one time. These ticks also cause an economic damage by direct blood feeding. In Egypt, El-Kamham et al. (2002) mentioned that birds may lose 56.7 kg. blood and 2.08 kg. plasma protein per year if only one pair of *A. persicus* or *A. hermanni* feed just once on each bird. In Pakistan, Khan et al. (2001) showed that a single *A. persicus* sucked 18.57 mg. blood daily and 0.06 g. per bird. They also estimated an annual loss resulting from tick feeding about 2.13 million rupees.

Integrated pest management requires accurate pest identification besides other factors such as population monitoring, biological and chemical control (Axtell, 1999). The genus *Argas* belongs to the Argasidae family. It is generally recognized as a monophyletic group, diagnosed in the nymphs and adults by the ventral, rather than anterior, position of the gnathosoma, the absence of a dorsal shield or scutum, and the shape and position of the spiracle (Kloppen, 1992). The differences between the larval stages of species of the genus *Argas* that infest birds in Egypt are not obvious and need further studies. Moreover, the larval stage is considered as the most dangerous one because it feeds on birds for a long time up to 6 days. This feeding behavior facilitates movement of this tick during trade movements. So, this study aims at clarifying the differences between larvae of argasid species on birds by using Scanning Electron Microscopy (SEM) and morphometric analysis.

Material and Methods

1 – Specimens of *Argas* Larvae. The specimens of *Argas* spp. (commonly on birds in Egypt) were collected from various localities and reared in laboratory to obtain their larvae. These species were *A. persicus* (Oken, 1818), from Faculty of Agriculture chicken farm, Cairo University, Giza (30°01’N, 31°13’E); *A. hermanni* Audouin, 1827, from Abo-Talib village, Nobaria, El-Behera governorate (30°45’N, 30°00’E) and *A. arboreus* Kaiser, Hoogstraal and Kohls, 1964, from heron nests, Zoological Garden, Giza (30°01’N, 31°13’E). Ticks were identified according to Kaiser et al., (1964), Hoogstraal et al., (1981) and Walker et al., (2003). Adults of each species were fed on pigeons and were incubated at 30°C and 75% Relative Humidity. Fed ticks were checked daily to obtain eggs. Eggs were placed in a new cup and incubated at the same condition until they hatched to larvae. One
week post hatching, larvae were placed in water at 70°C ± 10, washed with normal saline 0.9% KCl several times and preserved in 70% ethanol FAMADAS et al., (1997).

2 – PREPARATION FOR SCANNING ELECTRON MICROSCOPY. Larvae were well cleaned by overnight immersion in water-glycerol-KCl solution at 40°C HOMSHIER & SONENSHINE (1977). This solution composed of 96.6% (by weight) glycerol combined with 0.05% (by weight) of potassium chloride (KCl) and 3.35% (by weight) of distilled water BROYD & WHARTON, (1971). Specimens were washed in tap water again using the ultrasonic cleaner. Then they were taken through a graded series of alcohol/water (25%, 50%, 75% and 100% ethyl alcohol) remaining one hour in each dilution KERRANS et al., (1976). Following this, specimens were glued by their dorsal and ventral surfaces to the SEM stub, and were dried by the dryer (Blazer Union, F1-9496 Blazer/Fürstentun Liechtenstein), using liquid carbon dioxide. Specimens mounted on SEM stubs were coated with gold by using a S150A Sputter Coater. Coated larvae were examined by Scanning Electron Microscopy (SEM).

3 – PREPARATION FOR MORPHOMETRIC MEASUREMENTS. Specimens, preserved in 70% alcohol were put in lactic acid for 24 hrs without heating for clearing. Internal organs of larvae were removed with fine sharp needle under a dissecting microscopy after which the larvae were washed with distilled water. These larvae were taken through a gradual series of alcohol/water as above, transferred to 1:1 absolute alcohol: xylene for 5 minutes and mounted on clean slides using Canada Balsam. Slides were put on hot plate (30°C) for 48 hrs. Measurements for 10 specimens for each species were given in millimeters by using optical microscopy.

Many structures of Argas species were measured as follows: body length from apex of palpi to posterior end of idiosoma, body width between two lateral sides behind coxae III, dorsal shield length across longitudinal axis, dorsal shield width across transverse axis, hypostomal length from the apex of hypostome to the last denticle of the outer file posteriorly, palpal length resulting from length of segment I + II + III + IV, basis capitulum length from base of hypostome to posterior end of basis capituli dorsally and ventrally, basis capitulum width across the widest transverse axis. Distance between the first posthypos­tomal setae (Ph1) and Ph2 on basis capituli ventrally.

RESULTS

1 – BODY. The body of Argas larvae is subcircular (excluding capitulum). A. persicus was found with 26 pairs of setae dorsally; 4 anterolateral, 10 posterolateral (total of 14 dorsolateral), 2 anterosubmedian, 7 posterosubmedian (total of 9 submedian) and 3 median (Fig. 1A). Ventrally, A. persicus has 16 pairs of setae; 3 internal coxal, 3 circum anal, 1 anal and 9 posterolateral (Fig. 1B). The setation patterns of A. arboreus and A. hermanni resemble that of A. persicus ventrally. A. arboreus has 27 or 28 pairs of setae dorsally. They are distributed exactly as in A. persicus except for 1-2 additional median setae. The number of dorsolateral setae was 14 pairs as in A. persicus, but their arrangement differed from that in A. persicus, with 8 anterolateral and 6 posterolateral (Fig. 2A). A. hermanni was 23 pairs of dorsal setae. They arranged 7 anterolateral, 8 posterolateral (total of 15 dorsolateral), 5 posterosubmedian and 3 median. It thus differed from the other two species as follows: anterosubmedian setae are lacking, dorsolateral group with additional pair of setae, posterosubmedian group with 5 rather than 7 pairs, and median setae as in A. persicus (Fig. 2B). In general, the setae of A. arboreus and A. hermanni were larger in size than in A. persicus. The shape of the dorsal plate is oval and it is located centrally for all species. The dorsal plate of A. hermanni divided into distinct bulging cells. These cells were more shallow in A. arboreus while the pattern A. persicus resembles grooves rather than discrete cells (Fig. 1A and 2A, B).

There are no significant differences in length and width of A. persicus and A. arboreus, “length 0.899 and 0.916 mm, width 0.666 and 0.645 mm, respectively” A. hermanni was significantly smaller than other two species. Its length and width were 0.675 and 0.480 mm, respectively. No significant differences of the ratio of length to width were found between of them. Length/width were 1.35, 1.41 and 1.40 for A. persicus, A. arboreus and A. hermanni, respectively (TABLE 1). The length of dorsal plate of A. hermanni
Fig. 1: *Argas persicus*. A. — Body, dorsal view includes; al = anterolateral setae (1-4), pl = posterolateral setae (1-10), asm = anterosubmedian setae (1-2), psm = poserosubmedian setae (1-7), m = median setae (1-3) and dsh = dorsal shield or plate. B. — Body ventral view includes; ic = internal coxal setae (1-3), ca = circumanal setae (1-3), an = anal setae (1) and pl = posterolateral setae (1-9).

Fig. 2: Dorsal body view of *Argas arboreus* (A.) and *A. hermanni* (B); al = anterolateral setae (1-8 for A. and 1-7 for B), pl = posterolateral setae (1-6 for A. and 1-8 for B), asm = anterosubmedian setae (1-2 for A. and 0 for B), psm = poserosubmedian setae (1-7 for A. and 1-5 for B), m = median setae (1-4 for A. and 1-3 for B) and dsh = dorsal shield or plate.
was 0.211 mm followed by *A. arboreus* (0.188 mm) and *A. persicus* (0.178 mm). The dorsal plate of *A. arboreus* was wider (0.180 mm) than that of *A. hermanni* (0.169 mm) or *A. persicus* (0.160 mm). The length/width gradually decreased from *A. hermanni* (1.25) to *A. persicus* (1.15) to *A. arboreus* (1.04) (Table 1).

### Table 1: Morphometric of body, dorsal plate and hypostome of *Argas* larvae.

<table>
<thead>
<tr>
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<th>Measurements (mm) ± SD</th>
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<tr>
<td></td>
<td>Body 1</td>
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<tr>
<td></td>
<td>length</td>
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<tr>
<td><em>A. persicus</em></td>
<td>0.899 ± 0.042</td>
</tr>
<tr>
<td><em>A. arboreus</em></td>
<td>0.916 ± 0.051</td>
</tr>
<tr>
<td><em>A. hermanni</em></td>
<td>0.675 ± 0.016</td>
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<tr>
<td><strong>F. value</strong></td>
<td>116.11*</td>
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Table 1: Morphometric of body, dorsal plate and hypostome of *Argas* larvae.

2 - **Hypostome.** The anterior dental formula for *A. persicus*, *A. arboreus* and *A. hermanni* is 3/3, 3/3 and 4/4, respectively. Its is 2/2 for all species posteriorly. The denticle numbers per file are 7/6/2, 8/8/3 and 10/9/3/2 for *A. persicus*, *A. arboreus* and *A. hermanni*, respectively. The outer edges of corona on the apex of the hypostome are bluntly rounded or truncate in *A. persicus*, slightly rounded in *A. arboreus* and distinctly rounded in *A. hermanni*. The coronas have a varying number of small hooklets. In *A. persicus* these hooklets form two transverse rows (Fig 3 A-C). The hypostomes of *A. arboreus* and *A. hermanni* are approximately equal in length (0.152 and 0.151 mm) and significantly larger than that of *A. persicus* (0.130 mm) (Table 1).

3 - **Capitulum.** The chaetotaxy of the palpal segments is similar in *A. persicus* and *A. arboreus*; article I = 0, II = 5 (4 dorsal + 1 ventral), III = 4 (3 dorsal + 1 ventral) and IV = 4 (2 dorsal + 2 ventral) excluding the apical tuft setae (Fig. 4 A & B). Then, the number of setae per palp of *A. persicus* and *A. arboreus* was 13 pairs, 9 dorsally and 4 ventrally, but it was 14 pairs (9 dorsally and 5 ventrally) in *A. hermanni*. Larva of *A. hermanni* carry one extra seta ventrally on article III (Fig. 4 C). The apical tuft of article IV carried 8 setae in all the three species (Fig. 4 D). The chelicera are similar in all species, with 5 pairs of teeth; 3 separated laterally and 2 diffused together internally (Fig. 4 A-C). There are significant differences between the length of palps of these *Argas* species. Palps of *A. arboreus* are the longest (0.258 mm) followed by *A. persicus* (0.235 mm) and *A. hermanni* (0.197 mm) (Table 2).

The basis capitulum of the three *Argas* species is triangular shape dorsally (without setae) (Fig. 5 A) and rectangular ventrally. It carries two pairs of setae, post hypostomal setae (Ph1) anteriorly and post hypostomal setae (Ph2) posteriorly (Fig. 5 B). Basis capitulum dorsally is 0.077 to 0.083 mm long and 0.209 to 0.215 mm wide, without significant differences between species. However, ventral basis capitulum of *A. persicus* and *A. arboreus* is longer (0.130 and 0.132 mm) than that in *A. hermanni* (0.155 mm) (Table 2). The distance between setae Ph1 in *A.
**FIG. 3: Hypostomes and their coronae:**

A. *Argas persicus*  
B. *A. arboreus*  
C. *A. hermanni*

*arboreus* is significantly larger (0.080 mm) than in *A. persicus* (0.063 mm) or *A. hermanni* (0.050 mm). However, the distance between setae Ph2 of *A. arboreus* and *A. persicus* are approximately equal, 0.110 and 0.112 mm, respectively. It is significantly less in *A. hermanni* (0.100 mm). The distance between setae Ph2 to Ph1 in *A. persicus* and *A. arboreus* is similar 1.78 and 1.91, respectively. In *A. hermanni* that ratio is less (1.37) (Table 2).

**4- HALLER’S ORGAN AND SPIRACLE.** These structures were similar in all studied *Argas* larvae. Haller’s organ consists of an anterior pit and a posterior capsule (Fig. 5 C). Anterior pit included 7 sensilla located inside of it (Fig. 5 D). The spiracle has very small aperture, an oval shape, and is located between coxae II and III (Fig. 5 E).

**DISCUSSION**

The larval stage of the genus *Argas* is considered the most important stage because this stage stills on host to feeding for several days. As a result, it is playing role in distribution the tick from host to host or from place to place. There are three important species of soft tick infesting birds in Egypt; the chicken tick *A. persicus*, the heron tick *A. arboreus* and the pigeon tick *A. hermanni*. Publications dealing with morphological descriptions of these species very rare only (Sonenshine *et al.* , 1962 and Kaiser *et al.*, 1964). The first authors introduced a brief description for *A. persicus* and *A. hermanni* and the second authors described the larval stage of *A. arboreus* by light microscopy only. My results introduce more details about differentiation among them by the use more advancing procedures such as Scanning Electron Microscopy and additional morphometric differentiation.

The number of setae on dorsal surface were gradually decreased; 27 or 28, 26, and 23 in *A. arboreus*, *A. persicus* and *A. hermanni*, respectively. These setae were larger size in *A. arboreus* and *A. hermanni* than *A. persicus*. *A. arboreus* chaetotaxy resembles that in *A. persicus* except that *A. arboreus* carries 1-2 additio-
Fig. 4: Dorsal view of capitulum: A. — Argas persicus, B. — A. arboreus. C. — A. hermanni, D. — Apical tuft setae of Palps IV.
nal pairs of median setae. The number of lateral setae is equal in these two species (14 pairs) but they differ in distribution. *A. hermanni* differs from *A. persicus* by the following characters: no anterosubmedian setae, one additional pair of dorsolateral setae, and posterosubmedian setae with 2 pairs less. Chaetotaxy included 16 pairs ventrally for all species. The results of dorsal chaetotaxy agree with that recorded in (Sonenshine et al., 1962 and Kaiser et al., 1964). The ventral chaetotaxy of *A. persicus* and *A. hermanni* included 7 pairs (Sonenshine et al., 1962). Kaiser et al. (1964) showed that the chaetotaxy of *A. arboreus* was also 7 pairs. These differences with my finding of ventral setae may attribute to they do not calculate the 9 pair of setae ventro posterolaterally that were noticed by SEM. *A. persicus* and *A. arboreus* are
approximately equal in length and width, but *A. hermanni* is significantly smaller. Length of dorsal plate was larger in *A. hermanni* followed by *A. arboreus* and *A. persicus*. Dorsal plate of *A. hermanni* is divided into distinct bulging cells, which were more obvious in this species than in other species. The cells are more shallow in *A. arboreus* and diffused in *A. persicus*. Previous studies did not mention these characters before.

The dental formula of the hypostome was 3/3 in *A. arboreus* and *A. persicus* but in *A. hermanni* is 4/4. The number of denticles per file is highest in *A. hermanni* followed by *A. arboreus* and *A. persicus*. These results agree with (Sonenshine et al., 1962 and Kaiser et al., 1964) for *A. persicus* and *A. arboreus*. Although (Sonenshine et al., 1962) recorded that the dental formulae of *A. hermanni* was 3/3, my results reported 4/4, two outer files as (Sonenshine et al., 1962) and the inner files were two instead of one. The inner two files were well distinct by SEM. Corona is well rounded in *A. hermanni* and slightly rounded in *A. arboreus* and squared in *A. persicus*. Hypostome lengths for *A. arboreus* and *A. hermanni* were equal or larger than in *A. persicus*.

The chaetotaxy of the palpal segments, excluding the apical tuft setae on segment IV, comprises 13 pairs (9 dorsal and 4 ventral) in *A. persicus* and *A. arboreus*. One pair of setae is added on article III ventrally in *A. hermanni*. The apical tuft of palpal article IV includes 8 setae for all species. These results agree with (Sonenshine et al., 1962 and Kaiser et al., 1964) but the later authors noted that the apical tuft setae of article IV included 7 setae. This discrepancy may be because they depended on light microscopy. The palps of *A. arboreus* are the longest followed by *A. persicus* and *A. hermanni*. (This finding agree with (Sonenshine et al., 1962 and Kaiser et al., 1964).

Either dorsally or ventrally the basis capitulum showed the same morphological characters for all species except for the length of ventral capitulum of *A. hermanni* was lesser than others. The distance between posthypostomal setae (Ph1) of *A. arboreus* is the largest, followed by *A. persicus* and *A. hermanni*, but the distance between setae Ph2 is equal in *A. arboreus* and *A. persicus*. The ratio of Ph2 to Ph1 was equal in *A. arboreus* and *A. persicus* but less in *A. hermanni*. The measurements of Ph1 and Ph2 agree with (Sonenshine et al., 1962) in case of *A. persicus* and *A. hermanni* but no previous studies were recorded in *A. arboreus*. Morphological characters of Hal-ler’s organ and spiracle were the same for all species.

CONCLUSION

*A. persicus* larvae are identified as follows: with 26 pairs of setae dorsally (which are smaller than in *A. arboreus* and *A. hermanni*), equal in length and width to *A. arboreus* but larger than *A. hermanni*; length of dorsal plate less than in other species; dorsal plate with diffused cells that form grooves. Length of hypostome smaller than others. Dental formula of hypostome 3/3, the denticle number per file 7/6/2, corona of hypostome blunt, length of palps moderate, chaetotaxy of palpal segments with 13 pairs of setae excluding apical tuft setae, posthypostomal setae (Ph1) moderate, Ph2 equal with *A. arboreus* and larger than *A. hermanni*.

*A. arboreus* larvae are identified as follows: nearly to *A. persicus* than *A. hermanni*, with 27 or 28 pairs of setae dorsally (one or two pairs of median seta added than in *A. persicus*), equal in length and width with *A. persicus* and longer than *A. hermanni*, the length of dorsal plate moderate, dorsal plate with shallow cells, length of hypostome equal in *A. hermanni* and longer than in *A. persicus*, dental formula 3/3, denticle number per file 8/8/3, corona slightly rounded, length of palps larger than others, chaetotaxy exactly as *A. persicus*, Ph1 larger than in others, Ph2 equal in *A. persicus* and larger than in *A. hermanni*.

*A. hermanni* larvae was easy identified than others as follows: with lesser number of setae dorsally (23 pairs), without anterosubmedian setae, length of dorsal plate larger than in others, dorsal plate with distinct bulging cells, length of hypostome equal in *A. arboreus* and larger than in *A. persicus*, dental formula 4/4, denticle numbers per file 10/9/3/2, corona distinctly rounded, and Ph1 & Ph2 smaller than in others.

REFERENCES


