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Re- description of adult stages and description of immature stages of *Aceria tristriatus* (Nalepa, 1890), from western Iran

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Aceria tristriatus (Nalepa, 1890) is one of the major pests of walnut (Juglans regia L.: Juglandaceae) in Iran, especially in western of country. It causes galls on leaves that mainly concentrated along the main and lateral veins. The gall forms toward the upper surface of the leaf with the opening on the lower side. This paper reports the re-description of adult stages (protogyne, deutogyne and male) and description of immature stages of walnut leaf gall mite. Furthermore difference between all mobile stages in this species based on morphological characteristics is provided. Mites were collected from walnut trees infested with this pest in Hamedan and Lorestan provinces, western Iran

Key words: complementary description, different stages, walnut leaf gall mite, western Iran.

INTRODUCTION

The Eriophyoidea, known as the tetrapodili or four-legged mites, are worldwide in distribution (Walter *et al.* 2009). Eriophyoids were recognized as a taxon in the Acari by Siebold (1850). Fundamental knowledge on the morphology, biology, ecology, and economic importance of Eriophyoidea has been exhaustively compiled by Lindquist *et al.* (1996).

Most eriophyoid mite species are highly host-specific (Skoracka *et al.* 2010) and occur on various types of flowering plants, conifers, broad leaf trees, and shrubs (Oldfield, 1996). Eriophyoidea are an economically important group due to the direct damage they can cause to their hosts, their ability to transmit serious plant diseases, and also due to the possibility of using them as biological agents for weed control (Lindquist *et al.* 1996). Life cycle of Eriophyoid mites respectively contain: egg, two active immature instars and adult. The immature stages are sometimes referred to as larval or nymphal, or the first instar may be called a larva and second instar a nymph. In the adult stages of these mite two different forms of females (protogyne and deutogyne) and one form of male occur (Manson & Oldfield, 1996).

Aceria tristriatus firstly was described by Nalepa 1890, which described it as Phytoptus tristriatus. Farkas (1960) re– described female protogyne of this mite from Hungary and placed this species in genus Aceria. The walnut leaf gall mite is one of the major pests of walnut in western Iran (Khanjani & Ueckermann, 2007). This mite causes small and hard galls on upper side of the leaf with the opening on the lower side, that seen along the main and lateral veins, sometimes these galls are created on surface walnut fruit. Inside the galls all stages of this pest are found, deutogynes that are overwintering form of this species, in midsummer start to appear and at the end of the summer most of the population inside the galls are deutogyne. In the present paper immature stages of Aceria *tristriatus* (Nalepa, 1890) are described for first time; also due to being old the first description of adult stages that not refer to detail, this stages are re-described in this study.

MATERIAL AND METHODS

The specimens were collected from walnut (*Juglans regia* L.: Juglandaceae) leaves infested with leaf gall mite, *Aceria tristriatus* (Nalepa, 1890), in Hamedan and Lorestan provinces, Iran. The mites were mounted directly in Hoyer's medium on permanent microscope slides. The slides were dried in an oven (50°C), sealed with industrial painting material and examined with an Olympus BX51 Differential Interference Contrast (DIC) microscope. Drawings were made with a camera Lucida. For SEM pictures of deutogyne female, the mites were fixed in 3% buffered glutaraldehyde, dehydrated in graded ethanol, dried in a critical point dryer, sputter-coated with gold and examined in a JEOL JSM-840 field emission scanning electron microscope (SEM) operating at an accelerating voltage of 7 kV. All measurements are presented in micrometers (µm), according to the methodology of Amrine & Manson (1996) and de Lillo *et al.* (2010). The morphological terminology used follows Lindquist (1996).

RESULTS

Taxonomy Family Eriophyidae Nalepa, 1898 Subfamily Eriophyinae Nalepa, 1898 Tribe Aceriini Amrine & Stansy, 1994 Genus *Aceria* Keifer, 1944 *Aceria tristriatus* (Nalepa, 1890)

Species diagnosis: Opisthosoma wormlike, with annuli sub-equal dorsoventrally, in adult annuli with many micro tubercles dorsally and ventrally (dorso-opisthosomal annuli in deutogyne form without micro tubercles); prodorsal shield with obvious design in base and without anteromedian lobe; empodium simple, 3-rayed. Prodorsal shield tubercles on rear margin of shield, setae *sc* divergently backwards. Epigynum is smooth.

Difference between mobile stages: Immature instars consistently differ from adults in lacking genitalia and any rudiment of a genital opening. Larva is sluggish while nymph is more active, also this two immature can be distinguished with differences in sizes of structures, that larva is smaller than nymph. Females distinguished from males with differences in shape of genital region and size of body. Two form of females are differ by: Protogyne whitish to yellowish in color and dorso-ventrally opisthosomal annuli with microtubercles opposed to deutogyne form pink to reddish in color and dorso opisthosomal annuli without microtubercle and ventrally are reduced.

Protogyne (Figs. 1–7) (n= 10). Body is elongate and wormlike, whitish to yellowish in color; 220–234 long (from tip of the pedipalp to the end of the anal lobe), 54–58 wide at level of seta *c2*. Gnathosoma 23–24 long, 12–14 width, projecting obliquely down, pedipalp coxal seta (*ep*) 3–4 long, dorsal pedipalp genual seta (*d*) 4–5, cheliceral stylets 16–17 long. **Prodorsal shield** (Fig. 1) 29–32 long, 36–40 wide. Prodorsal shield with obvious design in base, consisting of one median line; two admedian lines and two incomplete submedian lines I. Subtriangular in shape, scapular tubercles on the rear shield margin, 4–5 long, 18–19 apart; scapular setae sc 26–30 long, divergently backwards. **Coxigenital plates** (Fig. 2) I and II smooth, coxigenital region whit one annuli without microtubercle; anterolateral setae on coxisternum I (*1b*) 6–7 long, 8–10 apart; setae *1a* 29–33 long, 5–6 apart; proximal setae on coxisternum II *2a* 45–49 long, 14–17 apart. Prosternal apodeme 9–10. **Legs** with all usual segments and setae. Leg I (Fig. 4) 29–30 (measured from trochanter to tarsus



FIGURES 1-2. A. tristriatus (Nalepa) (Female protogyne): 1. Dorsal view of protogyne; 2. Ventral view of protogyne.



FIGURES 3-7. *A. tristriatus* (Nalepa) (Female protogyne): 3. Lateral view of protogyne; 4. Leg I; 5. Leg II; 6. Empodium; 7. Internal genitalia.

excluding empodium) long; trochanter 4–5; femur 9–10 long, setae bv 11–13 long; genu 5 long, setae l'' 23–25 long; tibia 4–5 long, setae l' 3–4 long; tarsus 9–10 long, setae ft'' 22–25 long, setae ft' 11–12 long; setae u' 3 long; tarsal solenidion 8 long. Leg II (Fig. 5) 26–28 long; trochanter 4; femur 8–9 long, setae bv 12–13 long; genu 4–5 long, setae l'' 14–16 long; tibia 4 long; tarsus 7–8 long, setae ft'' 23–26 long, setae ft' 8–9 long; setae u' 3 long; tarsal solenidion 7–8 long. Tarsal empodium I and II 6–7 long, simple, with 3 rays (Fig. 6). **Opisthosoma** (Figs. 1–3) dorsally with 68–72 annuli, with round obscure microtubercles, six or seven annuli on front of opisthosoma with stretched microtubercles on ventral are narrow and elongated than dorsum, several posterior annuli and also along the middle of the opisthosoma with more stretched microtubercles. Setae c2 18–20 long, 43–47 apart on ventral annulus 7–8; setae d 17–19 long, 34–36 apart on ventral annulus19–21; setae e 16–18 long, 17–19 apart on ventral annulus 35–37; Setae f 19–21 long, 18–20 apart, on ventral annulus 5th ventral annulus from rear (Fig. 2); setae b1 9–10 long11–13 apart; Setae b2 101–125 long, 12–14 apart. **Genitalia** (Figs. 2,7) 13–14 long, 19–20 wide, epigynum smooth; setae 3a 8–10 long, 9–10 apart.

Material examined. Hamedan Province: Heydareh village (34° 48' N; 48° 28' E) – 4 Jul. 2016, 13 Aug. 2016; Shahrestaneh village (34° 70', N; 48° 35' E) – 11 Jul. 2016, 17 Sep. 2016; Ganjnameh (34° 45', N; 48° 26' E) - 12 Agu. 2016; Faculty of Agriculture of Buali Sina University (34° 48' N, 48° 29' E) – 18 Jun. 2016, 9 Aug. 2016, 20 Sep. 2016. Lorestan Province: Kahman canyon (33° 57' N, 48° 19' E) – 28 Jun. 2016, 1 Agu. 2016, 8 Sep. 2016; Aleshtar county (33° 32' N, 49° 27' E) – 28 Jun. 2016, 1 Agu. 2016, 8 Sep. 2016, Kakareza village (33° 43' N, 48° 15' E) – 25 Sep. 2016.

Deutogyne (Figs. 8–13) (n= 10). Body wormlike, pink to reddishin color; 188–209 long (from tip of the pedipalp to the end of the anal lobe), 45-51 wide. Gnathosoma 20-23 long, 10-12 width, projecting obliquely down, pedipalp coxal seta (p) 3 long, dorsal pedipalp genual seta (d) 4, cheliceral stylets 14-15 long. Prodorsal shield (Figs. 8, 2) 24-27 long, 31-35 wide. Prodorsal shield only in base with design that length of them shorter than female protogyne, consisting of one median line; two admedian lines and two incomplete submedian lines I. Subtriangular in shape, scapular tubercles on the rear shield margin, 4 long,17–18 apart; scapular setae sc 26–29 long, divergently backwards. Coxigenital plates (Fig. 9) I and II smooth, coxigenital region whit one annuli without microtubercle; anterolateral setae on coxisternum I (1b) 5-6 long, 8-9apart; setae 1a 30-33long, 6-7apart; proximal setae on coxisternum II 2a 47-51 long, 13-15 apart. Prosternal apodeme 8-9. Legs with all usual segments and setae. Leg I (Fig. 12) 29-30 (measured from trochanter to tarsus excluding empodium) long; trochanter 3-4; femur 8-10 long, setae by 9-11 long; genu 5 long, setae l" 20-24 long; tibia 5 long, setae l' 2-3 long; tarsus 9-10 long, setae ft" 23-26 long, setae ft' 9-11 long; setae u'2-3 long; tarsal solenidion 7-8 long. Leg II (Fig. 13) 26-27 long; trochanter 3-4; femur 7-9 long, setae by 9-11 long; genu 4-5 long, setae l" 11-13 long; tibia 4 long; tarsus 7-9 long, setae ft" 20-23 long, setae ft' 7-8 long; setae u' 2-3 long. Tarsal empodium I and II 6-7 long, simple, with 3 rays. Opisthosoma (Figs. 8-10), dorsally with 66-69 annuli, without microtubercle (Fig. 8). Ventral with 61-63 annuli, with faint and flattened microtubercles, those are decrease than female protogyne. Setae c2 10-13 long, on ventral annulus 6-7, 34-37 apart; setae d 10-13 long, on ventral annulus 19-20, 27-30 apart; setae e 11-12 long, on ventral annulus 34-36, 15-16 apart; Setae f 14-16 long, 18–19 apart, on ventral annulus 6th ventral annulus from rear (Figs. 9–10). setae h1 11–13 long 9-11 apart; Setae h2 110-135 long, 12-14 apart. Genitalia 10-12 long, 17-18 wide, epigynum smooth; setae 3a 5-6 long, 6-7 apart (Fig. 9).

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FIGURES 8-13. *A. tristriatus* (Nalepa) (Female deutogyne): 8. Dorsal view of deutogyne; 9. Ventral view of deutogyne; 10. Lateral view of deutogyne; 11. Prodorsal shield; 12. Leg I; 13. Leg II.

Material examined. Hamedan Province: Heydareh village (34° 48' N; 48° 28' E) –29 Aug. 2016, 6 Oct. 2016; Shahrestaneh village (34° 70', N; 48° 35' E) –17 Sep. 2016; Ganjnameh (34° 45', N; 48° 26' E) – 17 Nov. 2016, 1 Dec. 2016; Faculty of Agriculture of Buali Sina University (34° 48' N, 48° 29' E) – , 10 Sep. 2016, 21 Sep. 2016, 3 Oct. 2016, 14 Nov. 2016, 12 Dec. 2016, 27 Dec. 2016. Lorestan Province: Kahman canyon (33° 57' N, 48° 19' E) –8 Sep. 2016, 3 Oct. 2016; Aleshtar county (33° 32' N, 49° 27' E) –8 Sep. 2016, Kakareza village (33° 43' N, 48° 15' E) – 25 Sep. 2016.

Male (Figs. 14–18) (n= 8). Body is elongate and wormlike, whitish to yellowish in color; 186–198 long (from tip of the pedipalp to the end of the anal lobe), 48-52 wide. Gnathosoma 17-19 long, 10-11 width, projecting obliquely down, cheliceral stylets 12-14 long. Prodorsal shield (Fig. 14) 24-26 long, 29-31 wide. Prodorsal shield design is similar to female protogyne. Subtriangular in shape, scapular tubercles on the rear shield margin, 4 long, 14–16 apart; scapular setae sc17–19 long, divergently backwards. Coxigenital plates (Fig. 15) and coxigenital region similar to female. 1b 3 long, 8-10 apart; setae 1a 20-23 long, 6-7 apart; 2a 39-42 long, 11-14 apart. Prosternal apodeme 6-7. Legs with all usual segments and setae. Leg I (Fig. 17) 23-24 (measured from trochanter to tarsus excluding empodium) long; trochanter 3; femur 7-8 long, setae by 9-10 long; genu 4-5 long, setae l" 17–20 long; tibia 4 long, setae l' 2 long, tarsus 7 long, setae ft' 20–22 long, setae ft' 9–10 long; setae u' 2-3 long; tarsal solenidion 7-8 long. Leg II (Figs. 18) 20-21 long; trochanter 2-3; femur 6-7 long, setae bv 10-11 long; genu 4 long, setae l" 10-12 long; tibia 3-4 long; tarsus 6-7 long, setae ft" 17-20 long, setae ft' 6-7 long; setae u' 2 long; tarsal solenidion 6-7 long; tarsal empodium I and II 5 long, simple, with 3 rays. Opisthosoma dorsally (Figs. 14) with 61-64 annuli, with round obscure microtubercles, three posterior annuli without microtubercles. Ventral (Fig. 15) with 57-59 annuli, posterior annuli with more stretched microtubercles. Setae c2 10-11 long, on ventral annulus 6-7, 38–40 apart; setae d 10–12 long, on ventral annulus 16–18, 31–33 apart; setae e 9–10 long, on ventral annulus 30-32, 14-16 apart; Setae f11-13 long, 17-19 a part, on ventral annulus 5th ventral annulus from rear; setae h1 5-6 long 8-9 apart; Setae h2 61-68 long, 11-12 apart. Genitalia (Fig. 15) 16-18 wide, setae 3a 4-6 long, 10-11 apart.

Material examined. The locations and dates of collection of male, are similar to female protogyne.

Nymph (Figs. 19–23) (n= 7). Body is wormlike, whitish in color; 155–164 long (from tip of the pedipalp to the end of the anal lobe), 41-44 wide. Gnathosoma 16-17 long, 10-11 width, projecting obliquely down. Prodorsal shield (Fig. 19) 22-23 long, 29-31 wide. Subtriangular in shape scapular tubercles on the rear shield margin 3-4 long, 16-17 apart; scapular setae sc 14-17 long, divergently backwards. Prodorsal shield with short and incomplete lines consisting of one median line; two admedian lines and two submedian that are thinner than adult. Coxigenital plates I and II smooth; setae 1b 2 long, 7 apart; setae 1a 15-17 long, 6-7 apart; 2a 22-26 long, 13-14 apart. Legs with all usual segments and setae. Leg I (Fig. 22) 18-19 (measured from trochanter to tarsus excluding empodium) long; trochanter 2; femur 6 long, setae bv 6-7 long; genu 3 long, setae l" 14-16 long; tibia 3 long, setae l' 2 long; tarsus 5 long, setae ft" 14-15 long, setae ft' 5-6 long; setae u' 2 long; tarsal solenidion 5 long; tarsal empodium 4-5 long. Leg II (Fig. 23) 15-16 long; trochanter 2; femur 5 long, setae bv 5-6 long; genu 3 long, setae l" 5-6 long; tibia 3 long; tarsus 4-5 long, setae ft" 12-14 long, setae ft' 4-5 long; setae u' 2 long; tarsal solenidion 5 long; tarsal empodium 4 long. Tarsal empodium I and II simple, with 3 rays. Opisthosoma dorsally (Fig. 19) with 47-50 annuli, with round obscure microtubercles that are decrease than adults, four posterior annuli without microtubercles. Ventral (Fig. 20) with 44-46 annuli, only ten posterior annuli with microtubercles. Setae c2 9-10 long, on ventral annulus 7-9, 34-37 apart; setae d 9-10 long, on ventral annulus 17-19, 26–28 apart; setae e 8–9 long, on ventral annulus 27–28, 16–17 apart; Setae f 10–11 long, 17–19

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apart, on ventral annulus 4th ventral annulus from rear; setae *h*1 4 long 7 apart; Setae *h*2 55–58 long, 9–10 apart. External structure of genitalia absent, setae *3a* 2–3 long, 6–7 apart.

Material examined. The locations and dates of collection of egg and immature stages, are same with female protogyne.

Larve (Figs. 24-28) (n= 5). Body is wormlike, whitish in color; 114-122 long (from tip of the pedipalp to the end of the anal lobe), 34-39 wide. Gnathosoma 14-15 long, 9-10 width, projecting obliquely down. Prodorsal shield 18-20 long, 26-28 wide. Subtriangular in shape, scapular tubercles 3 long, 14–15 apart; scapular setae sc 11–12 long. Coxigenital plates I and II smooth; 1b 1 long, 6 apart; setae 1a 9-10 long, 5-6 apart; 2a 12-14 long, 12-13 apart. Legs with all usual segments and setae. Leg I (Fig. 27) 15–16 (measured from trochanter to tarsus excluding empodium) long; trochanter 2; femur 5 long, setae bv 4-5 long; genu 3 long, setae l" 11-12 long; tibia 3 long, setae l'1 long; tarsus 4 long, setae ft' 10-11 long, setae ft' 4-5long; setae u'1 long; tarsal solenidion 5 long. Leg II (Fig. 28) 13 long; trochanter 2; femur 4 long, setae by 3-4 long; genu 2 long, setae l'' 4-5 long; tibia 2 long; tarsus 3 long, setae ft" 9-10 long, setae ft' 3-4 long; setae u' 1 long; tarsal solenidion 5 long; tarsal empodium I and II 4 long, simple, with 3 rays. Opisthosoma dorsally (Fig. 24) with 44-46 annuli, with round obscure microtubercles that are decrease than nymph, two or three annuli on front and four posterior annuli of opisthosoma without microtubercles. Ventral (Fig. 25) with 35-39 annuli, seven posterior annuli with few microtubercles. Setae c2 7 long, on ventral annulus 6-7, 32-35 apart; setae d 7 long, on ventral annulus 15-16, 26-28 apart; setae e 6 long, on ventral annulus 23, 14-16 apart; Setae f 7-8 long, 15-16 apart, on ventral annulus 2th ventral annulus from rear; setae h1 3 long 4-5 apart; Setae h2 25-28 long, 7-8 apart. External structure of genitalia absent, setae 3a 1-2 long, 5-6 apart.

Egg and embryo (Figs. 29–30): The egg is ovoid in shape and 61–64 long, 39–41 width (Fig. 29). In figure 30 embryo showing through the eggshell.



FIGURES 14-18. A. tristriatus (Nalepa) (Male): 14. Dorsal view of male; 15. Ventral view of male; 16. Lateral view of male; 17. Leg I; 18. Leg II.



FIGURES 19-23. A. tristriatus (Nalepa) (Nymph): 19. Dorsal view of nymph; 20. Ventral view of nymph; 21. Lateral view of nymph; 22. Leg I; 23. Leg II.



FIGURES 24-30. A. tristriatus (Nalepa) (Larva, egg, embryonic): 24. Dorsal view of larva; 25. Ventral view of larva; 26. Lateral view of larva; 27. Leg I; 28. Leg II; 29. Egg; 30. Embryonic.

DISSCUSSION

In the first description of this species by Nalepa 1890, little information about morphology was provided. Farkas (1960) re-described female protogyne and briefly explained its characteristics, but not mention about other stages of this species. In current study, all details of morphology such as number of annuli, shape of microtubercles and measurements of setae in all stages are presented. Also difference between mobile stages is mentioned. Redescription of female protogyne in this study resemble re-described by Farkas (1960) but it differs by: length of body 220–234; c2 18–20; d 17–19; e 16–18; h2 101–125; in this study opposed to length of body 140; c2 14; d 14; e 12; h2 105 in prior study.

Re-description of deutogyne in this study resemble description by Flechtmann et al. (2002) in France; however, they have differences: 1. Iranian specimens (this study) with two incomplete admedian lines at base of prodorsal shield opposed to complete admedian lines in France specimens; 2. Each ray of empodium with one appendage versus with two appendages in France specimens.

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