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Taxonomy and systematics

Aspiculuris mexicana n. sp. (Nematoda: Heteroxynematidae) parasite of Cricetidae rodents from Mexico, with a taxonomic key for the species of the genus

Aspiculuris mexicana *n. sp. (Nematoda: Heteroxynematidae) parásito de roedores Cricetidae de México, con una clave taxonómica para las especies del género*

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Abstract

During November 2010, a tarabundí vole, *Microtus oaxacensis* Goodwin, 1966 and 2 Mexican harvest mice, *Reithrodontomys mexicanus* Sassure, 1860 collected in Oaxaca, Mexico, were examined for helminths. The nematode *Aspiculuris mexicana* n. sp., was found in the colon of both species. This parasite is characterized by 2 clear synapomorphies: *1)* cervical alae ending abruptly at the middle-length of esophageal bulb, and *2)* the presence of 16 caudal papillae with unique arrangement: 3 preanal pairs; 1 pair adanal; a single papilla immediately postanal; 2 postanal pairs; 1 single ventral papilla, and 1 ventral pair far away from the anus. In addition, this work presents an updated taxonomic key for 17 of the 20 valid species included in the genus.

Keywords: *Microtus oaxacensis*; *Reithrodontomys mexicanus*; Rodentia; Neotropics; Helminths

Resumen

Durante noviembre de 2010, un ejemplar del topillo *Microtus oaxacensis* Goodwin, 1966 y 2 del ratón cosechero mexicano *Reithrodontomys mexicanus* Sassure, 1860 colectados en Oaxaca, México, fueron examinados en busca de helmintos. El nemátodo *Aspiculuris mexicana* n. sp. fue localizado en el colon de ambas especies. Este parásito

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se caracteriza por 2 sinapomorfías claras: *1)* alas cervicales terminando abruptamente en la región media del bulbo esofágico y *2)* presencia de 16 papilas caudales con una distribución particular: 3 pares preanales; 1 par adanal; 1 papila media postanal; 2 pares postanales; 1 papila ventral y 1 par ventral lejos del ano. En este artículo se incluye una clave taxonómica actualizada para 17 de las 20 especies válidas del género.

Palabras clave: *Microtus oaxacensis*; *Reithrodontomys mexicanus*; Rodentia; Neotrópico; Helmintos

Introduction

Heteroxynematidae (Nematoda: Oxyuroidea) comprises a group of nematodes with a cosmopolitan distribution. The males in this family feature a tail with a regular shape, which is not bluntly truncated. Further, the males do not have long protruding papillae extending into cuticular alae and have genital papillae mainly in the perianal region (Petter & Quentin, 1976). Currently, this family contains 13 genera with more than 59 species parasites of birds and mammals (rodents and lagomorphs) (Hodda, 2011). In Mexico, Heteroxynematidae includes 3 genera: *Aspiculuris* Schulz, 1927 (2 species), *Lamotheoxyuris* Falcón-Ordaz, Fernández & García-Prieto, 2010 (1 species) and *Dermatoxys* Schneider, 1866 (2 species) (García-Prieto et al., 2012). *Aspiculuris* can be distinguished from other members of the family by having cuticular striations poorly marked and cervical alae well developed, extending from the cephalic vesicle (Petter & Quentin, 1976). The 19 nominal species of this genus known so far are parasites of rodents, including Cricetidae (Liu et al., 2012). This rodent family is cosmopolitan, with more than 130 genera and 680 species, from which 23 genera and 141 species are distributed in Mexico (Ceballos et al., 2005; Wilson & Reeder, 2005). As part of the authors commitment to inventory the helminth diversity associated with vertebrates, we found an undescribed species of the genus *Aspiculuris* which is herein described using light and scanning electron microscopy.

Materials and methods

In November 2010, 1 specimen of the tarabundi vole, *Microtus oaxacensis* Goodwin, 1966 and 2 of the Mexican harvest mouse, *Reithrodontomys mexicanus* Sassure, 1860 were collected at km 134 of the highway Oaxaca-Tuxtepec (17°25'10" N, 96°29'53" W), Oaxaca, Mexico. Hosts were collected under permission FAUT-0170 issued by Secretaría de Medio Ambiente y Recursos Naturales. Rodents were anesthetized by isoflurane inhalation, euthanized by cervical dislocation, and examined for endoparasites. Helminths were removed from the intestine and placed in 0.85% saline solution, fixed in hot 4% formaldehyde and stored in 80% ethanol. Nematodes were cleared with Amman's lactophenol and temporarily mounted for morphological study. Measurements, expressed in micrometers unless otherwise stated, are given as the mean, and followed by range and sample size in parentheses. Figures were drawn with the aid of a drawing tube. Two specimens for scanning electron microscopy (male and female) were dehydrated in a graded ethanol series, critical-point dried with CO_2, and then coated with a gold-palladium mixture. Specimens were examined with a Hitachi SU1510 electron microscope. Type specimens were deposited at Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, México City, Mexico.

Description

Aspiculuris mexicana n. sp.
http://zoobank.org/urn:lsid:zoobank.org:act:3115FA79-B9B3-4F10-82B8-2205D5F20C71

Diagnosis. Medium size nematodes. In both sexes, cervical alae begin at the prominent cephalic vesicle and end abruptly at the middle-length of esophageal bulb (Fig. 1) forming an acute angle (Fig. 2a, b). Triangular mouth with a small opening surrounded by 6 reduced lips. Four single, large cephalic papillae: 2 dorso and 2 ventro-laterals. Two prominent amphids between each pair of papillae (Fig. 3a).

Male (based on holotype, 2 paratypes, one of them studied under SEM). Body length 2.81 mm (2.21-3.42; n = 2), width at middle body 110 (80-130; n = 2); cephalic

Figure 1. Light micrograph of *Aspiculuris mexicana* n. sp. showing cervical ala extension.

vesicle length 65 (60-70; n = 2) by 70 (50-80; n = 2) width. Esophagus length 340 (310-370; n = 2). Esophageal bulb 95 (90-100; n = 2) length by 60 (50-70; n = 2) width. Nerve ring 100 (90-110; n = 2) from anterior end; excretory pore not observed. Cervical alae 270 (210-330; n = 2) length, begins at 40 (30-50; n = 2) from the anterior end and 20 (n = 1) from the lateral alae; length of lateral alae 3.09 mm (n = 1). Tail 190 (180-210; n = 2) curved ventrally, with pointed tip, provided with 3 pairs of alae (Fig. 3b): 1 preanal and 2 postanal (lateral and sublateral), not enclosing the caudal apex (Figs. 2c, d). The 16 caudal papillae are arranged as follows: 3 preanal pairs (1 subventral and 2 medio-ventral pairs near to the anus); 1 adanal pair; a single papilla immediately postanal; 2 postanal pairs (1 subventral pair, 1 smaller ventral pair); 1 single ventral papilla, and 1 ventral pair far away from the anus (Figs. 2d, e; 3b). Two postero-lateral fasmids situated posterior to the last pair of papillae.

Female (based on alotype and 1 paratype). Body length 5.85 mm (5.69-6.02; n = 2), width at middle body 250 (220-270; n = 2). Cephalic vesicle 85 (80-90; n = 2) length by 125 (120-130; n = 2) width. Esophagus 310 (290-320; n = 2) length. Esophageal bulb 110 by 90 (n = 1). Nerve ring and excretory pore 145 (140-150; n = 2), and 950 (890-1010; n = 2) from anterior end, respectively. Cervical alae begin 25 (20-30; n = 2) from the anterior end (Fig.

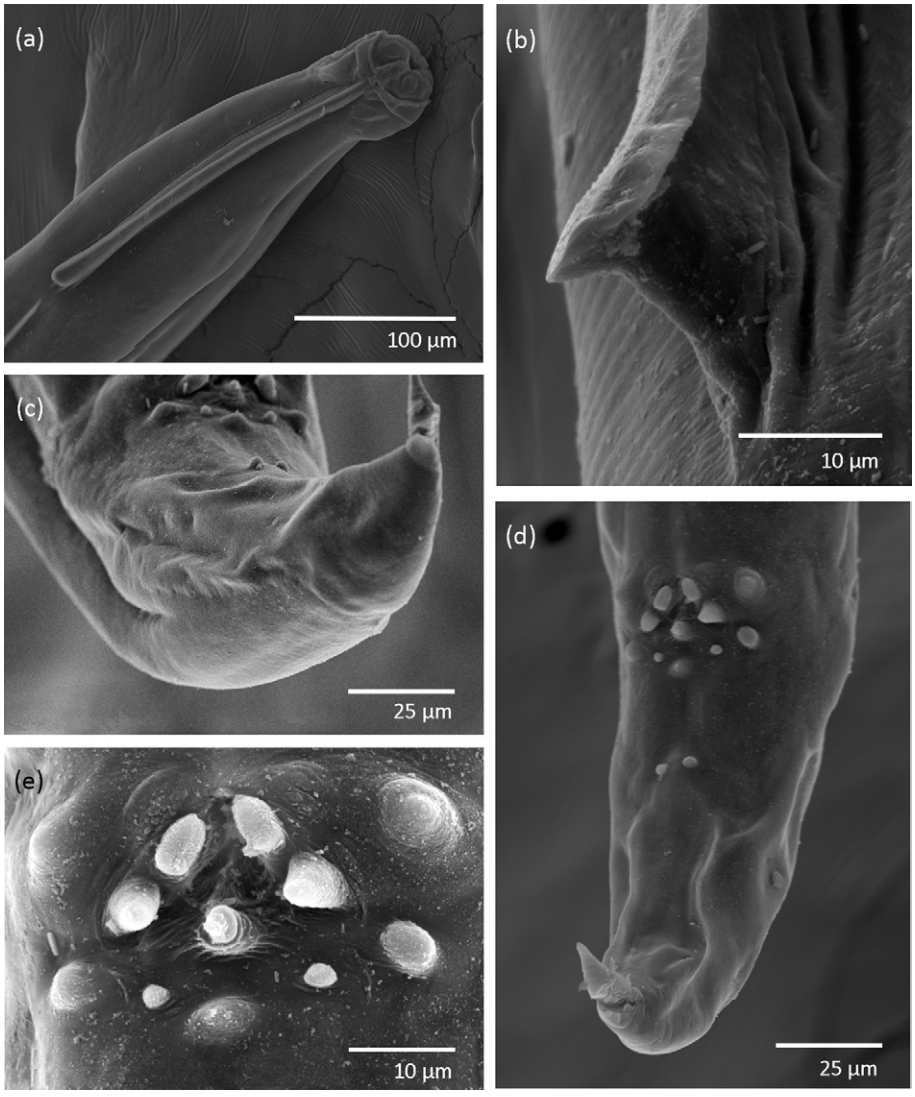

Figure 2. Scanning electron micrographs of *Aspiculuris mexicana* n. sp. (a) Male, anterior end, showing cervical ala extension; (b) cervical ala; (c) male, caudal end, lateral view; (d) male, caudal end, showing papillae arrangement: preanal (1PrA, 2PrA, 3PrA), adanal (1AA) and postanal (1PA, 2PA, 3PA, 4PA, 5PA); (e) male, papillae arrangement around anus.

3C); length 395 (390-400; n = 2). Pre-equatorial vulva 2.15 mm (2.04-2.26; n = 2) from anterior end (Fig. 3d). Tail 940 (n = 1). Eggs 74 (60-90; n = 10) by 39 (30-40; n = 10) (Fig. 3e).

Taxonomic summary

Type host: Tarabundi vole, *Microtus oaxacensis* Goodwin, 1966 (Cricetidae: Arvicolinae).

Additional host: Mexican harvest mouse, *Reithrodontomys mexicanus* Sassure, 1860 (Cricetidae: Neotominae).

Type locality: Oaxaca: km 134 of the highway Oaxaca-Tuxtepec (17°25'10" N, 96°29'53" W), Mexico.

Site of infection: colon.

Prevalence (%) *and mean intensity* (MA) *of infection*: *M. oaxacensis*: 100%; MA: 6. *Reithrodontomys mexicanus*: 50%; MA: 1.

Material deposited: CNHE 8658 (holotype male); CNHE 8659 (1 paratype, male; 1 paratype female); 8660 (alotype, female); CNHE 8661 (1 voucher, female).

Etymology: the parasite is named after Mexico, the country where the specimens were collected.

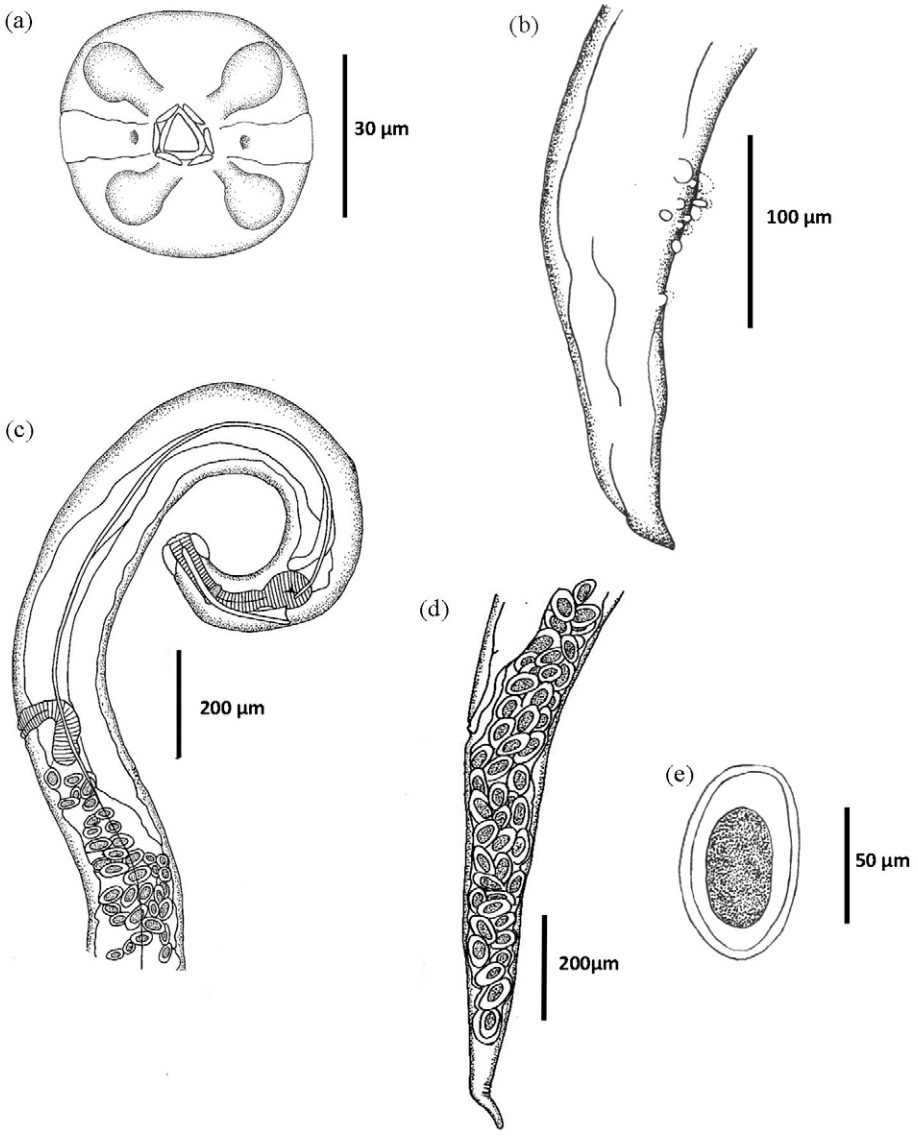

Figure 3. *Aspiculuris mexicana* n. sp. (a) Male, apical view; (b) male, caudal end; (c) female, anterior end; (d) female, caudal end; (e) egg.

Remarks

Aspiculuris is currently composed by 19 species parasites of rodents worldwide (Falcón-Ordaz et al., 2010; Liu et al., 2012). Quentin (1975) divided this genus in 2 groups based on the shape of the cervical alae. The first group includes 14 species with cervical alae that are interrupted at the level of the esophageal bulb and have an arrow shape-like end (Table 1). The second group

Table 1

Selected features of the *Aspiculuris* species with cervical alae interrupted at level of the esophageal bulb (measurements in millimeters).

Aspiculuris spp.	Female length	Male length	CA/EB	CP number	CA/T	Eggs size	Host family	Distribution	Reference
A. americana	3.17-3.88	2.25 - 2.46	Posterior	8	No	0.08 × 0.03	Cricetidae	Nearctic	Erickson (1938)
A. arianica	4.0	3.3	Anterior	11	No	-	Sciuridae	Palearctic	Kotrla & Daniel (1970)
A. aserbaidjanica	4.3 - 4.62	-	Posterior	-	-	0.09 - 0.10 × 0.04 - 0.05	Cricetidae	Palearctic	Tarzhimanova (1959)
A. dinnicki	8.0 - 8.25	3.86	Middle	10	No	0.11 × 0.05	Cricetidae	Palearctic	Schulz (1927)
A. huascaensis	3.22	2.48	Middle	12	No	0.06 ×0.02	Muridae	Nearctic	Falcón-Ordaz et al. (2010)
A. kazakstanica	< 6	-	Posterior	9	-	0.09 × 0.04	-	Palearctic	Miller & Schmidt (1982)
A. lahorica	-	-	Middle	10	-	0.07 - 0.08 x 0.03 - 0.04	-	Palearctic	Miller & Schmidt (1982)
A. mexicana	5.85	2.81	Middle	16	No	0.07 × 0.04	Cricetidae	Neotropic	Present study
A. pakistanica	< 6	-	Posterior	-	No	-	-	Palearctic	Miller & Schmidt (1982)
A. rysavyi	6-8	5-5.8	Posterior	11	Yes	0.08 × 0.04	Cricetidae	Palearctic	Kotrla & Daniel (1971)
A. schulzi	2.4 - 3.6	2.2	Anterior	10	-	0.08 × 0.04	Muridae	Palearctic	Cambieri (1957)
A. tetraptera	3.8 (3.5-4.5)*	0.7	Middle	10 + 2 double	Yes	0.09 × 0.04	Muridae	Neotropic	Hugot (1980)
A. tianjinensis	6.46	4.69	Posterior	12	No	0.09 × 0.05	Cricetidae	Palearctic	Liu et al. (2012)
A. tschertkovi	3.82 - 5.31	2.51 - 2.55	Middle	16	Yes	0.08 - 0.06 x 0.03 - 0.04	Cricetidae	Palearctic	Tarzhimanova (1959)
A. versterae	1.91	1.59	Anterior	10 + 1 double	Yes	0.09 × 0.04	Muridae	Ethiopian	Hugot (1980)

CP = Caudal papillae number; CA/EB = cervical alae ending level respect to esophagus bulb; CA/T = caudal alae enclosing or not the tail; *measurements after Behnke et al. (2015).

encompasses 5 species, in which the width of the alae decreases progressively and connects with the lateral alae (Table 2). The specimens described herein belong to the first group, which includes *Aspiculuris tetraptera* (Nitszch, 1821), *A. dinnicki* Schulz, 1927, *A. schulzi* Popov and Nasarova, 1930, *A. kazakstanica* Nasarova and Sweschnikova, 1930, *A. americana* Erickson, 1938, *A. lahorica* Akhtar, 1955, *A. pakistanica* Akhtar, 1955, *A. tschertkovi* Tarzhimanova, 1969, *A. aserbaidjanica* Tarzhimanova, 1969, *A. arianica* Kotrla and Daniel, 1970, *A. rysavyi* Kotrla and Daniel, 1971, *A. versterae* Hugot, 1980, *A. huascaensis* Falcón-Ordaz, Pulido-Flores and Monks, 2010 and *A. tianjinensis* Liu, Bu and Zhang, 2012 (Falcón-Ordaz et al., 2010; Liu et al., 2012). Based on the number of caudal papillae, *Aspiculuris mexicana* n. sp. can be differentiated from *A. americana*, *A. arianica*, *A. dinnicki, A. huascaensis, A. kazakstanica*, *A. lahorica*, *A. tetraptera*, *A. rysavyi*, *A. schulzi*, *A. tianjinensis* and *A. versterae*, because they have a smaller number (Table 1). In addition, cervical alae in the new species are abruptly interrupted at mid-length of esophageal bulb while in *A. schulzi*, *A. arianica* and *A. versterae* alae end in the pre-bulb region and in *A. americana*, *A. dinnicki*, *A. kazakstanica*, *A. rysavyi* and *A. tianjinensis* cervical alae surpass the esophageal bulb. The smaller body size of females in *A. huascaensis* and *A. tetraptera* allow us to separate them from *A. mexicana,* which measures 5.85 mm (Table 1).

The morphological features of 2 of the 3 remaining species (*A. aserbaidjanica* and *A. pakistanica*) are poorly known, even males of the first species are unknown. Nonetheless, in *A. aserbaidjanica*, the cervical alae extend beyond the posterior region of esophageal bulb (Tarzhimanova, 1969) and in *A. pakistanica* cervical alae end in the posterior region of this structure (Miller & Schmidt, 1982), whereas in the species herein described, the cervical alae end at mid-length of esophageal bulb. In addition, *A. pakistanica* and *A. aserbaidjanica* are distributed in the Palearctic region meanwhile *A. mexicana* is a species distributed in the transition zone between the Nearctic and Neotropical realms. Considering the number of caudal papillae, *A. tschertkovi* most closely resembles *A. mexicana* because both species have 16 papillae. Nevertheless, arrangement of caudal papillae of both species enables their differentiation: *A. tschertkovi* has 2 preanal papillae pairs, 2 adanal pairs and 4 postanal pairs (Tarzhimanova, 1969); on the other hand, in *A. mexicana* caudal papillae are disposed as follows: 3 preanal pairs; 1 adanal pair; 1 single papilla immediately postanal; 2 postanal pairs; 1 single ventral papilla, and 1 ventral pair. In addition, cervical alae end at different level in both species (Tarzhimanova, 1969) and their geographic distribution is distinct (Palearctic and Neotropical zone, respectively).

We considered that the specimens described herein correspond to a new species based on the morphological and morphometric features described in this study, particularly the extension of the cervical alae, and the number of caudal papillae and their particular distribution. *Aspiculuris mexicana* n. sp. is the twentieth species described for the genus and the fourth recorded in Mexico (Pulido-Flores et al., 2019). To the best of our knowledge, this study constitutes the first record of a helminth parasitizing both species of mammals: *M. oaxacensis* and *R. mexicanus*.

Behnke et al. (2015) analyzed the molecular phylogeny and morphology of 5 species of *Aspiculuris* parasites of voles and house mice. They concluded that the use of DNA is the most informative way to distinguish between species which are very similar to one another since number and configuration of caudal papillae are

Table 2

Selected features of *Aspiculuris* species with non-interrupted cervical alae (measurements in millimeters).

Aspiculuris spp.	Female length	Male length	CP number	CA/T	Eggs size	Host family	Distribution	Reference
A. asiatica	8.61-9.71	5.40	10 + 1 double	Yes	0.08 × 0.04	Muridae	Palearctic	Schulz (1927)
A. africana	3.2-4.2	2.3-3.6	10 + 1 double	No	0.09 × 0.05	Muridae	Ethiopian	Quentin (1966)
A. ratti	3.1-3.25	-	Unknown	Yes	0.09 × 0.04	Muridae	Oriental	Johnson (1969)
A. witenbergi	8.1	4.4	11 (1 pair is raspberry) + 1 double	No	0.07 × 0.04	Muridae	Palearctic	Quentin (1975)
A. shikoloueta	5.33	3.30-3.50	12 (1 pair is raspberry) + 1 double (possibly)	No	0.09 × 0.06	Muridae	Ethiopian	Inglis et al. (1990)

CP = Caudal papillae; CA/T = caudal alae enclosing or not the tail.

not reliable. We partially agree with this statement since only 5 of the 20 species of the genus have been studied from a molecular perspective (Behnke et al., 2015; Goswami et al., 2015). In addition, the identification of *Aspiculuris* is not based exclusively on the number and arrangement of the caudal papillae, but on a combination of morphological characters additional to these, such as the terminal region of the cervical alae with respect to the bulb of the esophagus, size of the eggs, length of the male and female body, tail enclosed or not by caudal alae, among others (Falcón-Ordaz et al., 2010; Liu et al., 2012). Based on these morphological data, in this work is presented an updated taxonomic key for 17 of the 20 species of *Aspiculuris* described to date, which can be well differentiated using a combination of morphological features.

Identification key for species of *Aspiculuris*.
(*A. aserbaidjanica*, *A. kazakstanica* and *A. pakistanica*, whose morphological descriptions are incomplete, are not included):

1a. Cervical alae interrupted at level of esophageal bulb with an arrow shape-like end 2
1b. Width of the cervical alae progressively decreasing, connected with lateral alae 16
2a. Cervical alae end anterior or at middle of esophageal bulb 3
2b. Cervical alae end posterior to esophageal bulb 11
3a. Caudal region with 10-12 papillae 4
3b. Caudal region with 14-16 papillae 10
4a. Caudal region with double papillae 5
4b. Caudal region with a single papilla 6
5a. Caudal region with 10 single and 1 double papillae . . . . . *A. versterae* Hugot, 1980
5b. Caudal region with 10 single and 2 double papillae . . . . . *A. tetraptera* (Nitzch, 1821)
6a. Palearctic distribution 7
6b. Nearctic distribution . . . . . *A. huascaensis* Falcón-Ordaz et al. 2010
7a. Caudal region with 10 papillae 8
7b. Caudal region with 11 papillae . . . . . *A. arianica* Kotrla and Daniel, 1970
8a. Egg size ranges 0.07 - 0.08 × 0.03 - 0.04 mm 9
8b. Eggs size 0.11 × 0.05 mm. . . . . *A. dinnicki* Schulz, 1927
9a. Cervical alae ending anterior to esophageal bulb . . . . . *A. schulzi* Popov and Nasarova, 1930
9b. Cervical alae ending at middle of esophageal bulb . . . . . *A. lahorica* Akhtar, 1955
10a. Tail enclosed by caudal alae; Palearctic distribution . . . . . *A. tschertkovi* Tarjymanova, 1959
10b. Tail not enclosed by caudal alae; Neotropical distribution . . . . . *A. mexicana* n. sp.
11a. Body size of female > 6 mm; Palearctic distribution 12
11b. Body size of female < 6 mm; Nearctic distribution . . . . . *A. americana* Erickson, 1938
12a. 11 caudal papillae; caudal alae enclosing tail . . . . . *A. rysavyi* Kotrla and Daniel, 1971
12b. 12 caudal papillae; caudal alae not enclosing tail . . . . . *A. tianjinensis* Liu et al., 2012
16a. Body size of female > 8 mm; Palearctic distribution 17
16b. Body size of female < 8 mm; Oriental or Ethiopian distribution 18
17a. Caudal area with 2 raspberry-like structures . . . . . *A. witenbergi* Quentin, 1975
17b. Caudal area with a double papillae . . . . . *A. asiatica* Schulz, 1927
18a. Tail not enclosed by caudal alae; Ethiopian distribution 19
18b. Tail enclosed by caudal alae; Oriental distribution . . . . . *A. ratti* Johnston, 1969
19a. Caudal area without raspberry-like structures . . . . . *A. africana* Quentin, 1966
19b. Caudal area with 2 raspberry-like structures . . . . . *A. shikoloueta* Inglis et al., 1990

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