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An identification key to species in the *mali* complex of *Aphelinus* (Hymenoptera, Chalcidoidea) with descriptions of three new species

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Abstract

The *Aphelinus mali* complex consists of eleven described species. Monophyly of this complex is well supported by a combination of traits: (1) a single complete row of setae proximal to the linea calva of the fore wing, with a few additional setae in the angle between this row and the marginal vein; (2) linea calva open (no setae at its posterior edge); (3) head and body dark except for parts of the metasoma; (4) meso- and metacoxae dark; (5) metafemur pale, (6) metatibia dark. Species within the complex have been distinguished by color and shape of antennal segments (particularly the third funicular segment), color of legs and metasoma, and relative length of ovipositor versus mesotibia. We provide a key for identifying species in the *mali* complex, and describe three new species, *Aphelinus glycinis* **sp. n.**, *Aphelinus rhamni* **sp. n.**, and *Aphelinus coreae* **sp. n.** from material in laboratory cultures originally reared from soybean aphid in China and Korea as candidates for biological control of soybean aphid, *Aphis glycines*.

Keywords
cryptic species, taxonomy, biological control

Introduction

The genus *Aphelinus* (Hymenoptera: Aphelinidae) comprises 84 recognized species (Noyes 2011), all of which are internal parasitoids of aphids. There are several complexes of closely related species in the genus, and identification of species within these complexes has been problematic (Heraty et al. 2007), leading to a confused literature on host specificity. The *Aphelinus mali* complex comprises 11 described species (Ashmead 1888; Evans et al. 1995; Gahan 1924; Girault 1913; Haldeman 1851; Hayat 1998; Prinsloo and Neser 1994; Timberlake 1924; Yasnosh 1963; Zehavi and Rosen 1988), and there are 6 other closely related species (Carver 1980; Hayat 1998; Howard 1917; Kurdjumov 1913; Walker 1839; Yasnosh 1963) that differ from the members of the complex in one or two traits (Table 1). The species within the complex have diverged little in morphology so the taxonomy within the complex has been confused, and many specimens have been identified as *A. mali* (Haldemann) or *A. gossypii* Timberlake that are likely different species based on differences in the aphid host species and geographical regions from which they were collected. We provide a key to identification of species in the *A. mali* complex based on 19 traits coded primarily from species descriptions. We describe three new species that were collected in China and Korea during exploration for natural enemies of the soybean aphid, *Aphis glycines* Matsumura, all of which are candidates for biological control of this important pest.

The genus *Aphelinus* consists of several complexes of cryptic species including the *mali* complex, the *varipes* complex (Heraty et al. 2007), the *asychis* complex (Kazmer et al. 1995, 1996), the *perpallidus* complex (unpublished data), and possibly others. Cryptic species are closely related species that differ little in the morphological features used for taxonomy, but differ critically in physiological, behavioral and ecological traits, such as climatic adaptation and host range (Darling and Werren 1990; DeBach 1969). Recent evidence from molecular studies suggests that cryptic species of hymenopteran parasitoids may be far more common than previously realized (Campbell et al. 1993; Clarke and Walter 1995; Darling and Werren 1990; Kankare et al. 2005a; Kankare et al. 2005b; Kazmer et al. 1996; Molbo et al. 2003; Rincon et al. 2006; Stouthamer et al. 2000; Stouthamer et al. 1999). The success of biological control programs depends on accurate species-level identifications of hosts and natural enemies, but choosing the best parasitoids for biological control programs is complicated by cryptic species (Rosen 1986; Wharton et al. 1990). Because cryptic species are difficult to recognize, studies on host ranges of parasitoids have often confounded more than one parasitoid species (Clarke and Walter 1995; Hopper et al. 1993), making analysis and prediction of host range difficult. Heightened concern about potential impacts of introduced parasitoids on non-target species makes accurate prediction of host range crucial to biological control introductions.

Table 1. Species in *Aphelinus mali* complex and related species

Group	Species	Original description
mali complex	*basilicus*	Hayat 1998
	campestris	Yasnosh 1963
	coreae sp. n. [1]	
	engaeus	Prinsloo and Neser 1994
	ficusae	Prinsloo and Neser 1994
	glycinis sp. n. [1]	
	gossypii	Timberlake 1924
	mali	Haldeman 1851
	niger [2]	Girault 1913
	paramali	Zehavi & Rosen 1989
	rhamni sp. n. [1]	
	sanborniae	Gahan 1924
	siphonophorae [2]	Ashmead 1888
	spiraecolae	Evans et al. 1995
related species	*chaonia* [3]	Walker 1839
	prociphili[3]	Carver 1980
	sharpae [3]	Hayat 1998
	brunneus [4]	Yasnosh 1963
	daucicola [4]	Kurdjumov 1913
	lapisligni [4]	Howard 1917

1 new species described in this paper

2 insufficient description to be included in tree or key

3 difference from *mali* complex: more than 1 line of setae in delta region

4 difference from *mali* complex: posterior femur dark

Methods

Three new species in the *A. mali* complex were collected from *Aphis glycines* in the Peoples Republic of China near Beijing and Xiuyan (Liaoning Province) and in the Republic of South Korea near Miryang (Gyeongsangnam Province) and maintained as laboratory cultures at the Beneficial Insects Introduction Research Unit, USDA-ARS, Newark, DE. All of the specimens described below were taken from lab cultures, killed in 95% ethanol, and most were critical-point-dried and card-mounted. Selected specimens were then slide-mounted in Canada balsam. Specimens photographed for coloration (Figs 1–6,15–20, and 29–34) were killed in ethanol and photographed as soon as possible, by placing specimens on a layer of KY® jelly in a small watch glass, submerging the specimen in ethanol, and photographing using a Leica MZ 16 stereomicroscope, fiber optic illumination, a Zeiss Axiomat MRc5 camera, and Helicon Pro image-stacking software. Slide-mounted specimens were photographed using differential interference contrast optics (DIC) with an Olympus BH2 compound microscope, and the same camera and software. Final modifications to images were made using Adobe Photoshop, Adobe Lightroom, and Adobe InDe-

sign. Type material and other specimens examined have been deposited as indicated in the species descriptions. The label data for each specimen has been digitized and all specimens bear individual accession numbers for Texas A&M University Insect Collection (e.g. TAMU x0616203), as well as a machine-readable bar-code. In the verbatim label data provided for holotypes, a single | symbol indicates a new line on a label, and the || symbol indicates a second or third label. Vouchers are maintained at -20°C in molecular grade ethanol at the Beneficial Insect Introduction Research Unit, Newark, Delaware, and at the Department of Entomology, Texas A&M University, College Station, Texas.

We tabulated and coded 19 traits for species in the *A. mali* complex, using the original species descriptions for the most part. These traits included color of scape, pedicel, club, coxae, femora, tibiae, tarsi, and metasoma, as well as shape of third funicle and club (length:width) and length of ovipositor relative to mesotibia. For some traits, males and females differed (e.g., F3 shape, procoxae color) and the values were scored separately. When trait data were lacking from original descriptions, we used data from later descriptions. Trait values for the new species in the complex were taken from specimens freshly killed in ethanol and slide-mounted specimens. These traits were used to construct an on-line, interactive, multiple entry identification key to the *mali* complex which is available on request. Of the 19 traits, 12 proved to be most consistent and useful in distinguishing species, and these are presented in Table 2.

Table 3 is a list of anatomical terms used in the paper followed by URI values (uniform resource identifiers), that will link the terms to precise definitions and illustrations in the Hymenoptera Anatomy Ontology project (see http://portal.hymao.org and http://hymao.org for more information on this initiative). Additional information on morphological terminology in Chalcidoidea is available in Gibson (1997) and http://www.canacoll.org/Hym/Staff/Gibson/apss/chglintr.htm.

The ventral surface of the antennal scape refers to the surface that is ventral when the antennae are deployed, or anterior when the antennae are folded on the face. F1, F2 and F3 refer to the first, second and third segments of the funicle of the antennal flagellum, respectively. T1, T2 etc. refer to metasomal terga. We use the term ovipositor to refer to the anatomical cluster consisting of the first valvula, second valvula, third valvula, first valvifer and second valvifer. Length of the ovipositor is the measurement (generally of a slide-mounted specimen) from the anterior margin of the second valvifer to the posterior (distal) end of the third valvula.

Measurements were made with an eyepiece reticle in a Leica MZ16 microscope or Zeiss standard 16 compound microscope, or from digital images captured using the methods described above. As with any species of *Aphelinus*, users will require series of high quality specimens, both male and female, and both card- or point-mounted and slide-mounted specimens, to obtain confident identifications. Noyes (1982) remains the best guide to techniques for preparing specimens of Chalcidoidea, and we have largely followed his recommendations for card-mounting and slide-mounting specimens.

Table 2. Traits coded for species in *mali* complex of *Aphelinus*.

Species	F3 female[1]	F3 male[2]	Club female[3]	Club male[4]	Procoxa color[5]	Profemur color[6]	Mesofemur color[7]	Protibia color[8]	Mesotibia color[9]	Metatibia color[10]	Metasoma color[11]	Ovipositor to mesotibia[12]
basilicus	1	2	2	2	1	1	1	1	2	1	1	1
campestris	1	1	1	1	1	2	1	1	2	1	2	?
coreae sp. n.	1	1	2	2	1	4	1	3	5	3	2	2
engaeus	1	2	2	2	2	3	4	2	4	3	5	2
ficusae	2	3	2	2	2	3	3	2	3	3	1	1
glycinis sp. n.	2	3	2	2	3	3	4	3	2	3	6	2
gossypii	1	1	1	1	1	2	1	1	1	1	2	2
mali	1	2	2	2	1	2	5	2	1	1	1	3
paramali	2	2	2	2	1	5	2	2	2	2	3	1
rhamni sp. n.	1	1	2	2	1	2	1	1	2	3	2	2
sanborniae	2	3	?	?	1	1	1	4	2	1	4	?
spiraecolae	1	1	1	1	1	3	4	2	4	1	1	3

1- F3 female: 1 = subquadrate, 2 = at least 1.2× longer than wide

2- F3 male: 1 = subquadrate, 2 = 1.2 to 2.0× longer than wide, 3 = more than 2.0× longer than wide

3- Club female: 1 = less than 2.5× as long as wide, 2 = at least 2.5× as long as wide

4- Club male: 1 = less than 3× as long as wide, 2 = at least 3× as long as wide

5- Procoxae: 1 = dark, 2 = yellow, 3 = pale in females, dark grey in males

6- Profemora: 1 = dark, 2 = dark with apex pale, 3 = yellow or pale, 4 = yellow in females, middle part fuscous in males

7- Mesofemora: 1 = dark, 2 = dark with apex pale, 3 = yellowish white, 4 = yellow in females, middle part fuscous in males, 5 = middle part dark brown in both sexes

8- Protibia: 1 = pale brownish yellow with basal half dark brown, 2 = white, yellow or pale, 3 = pale to grey, 4 = pale yellow, often with middle part darker

9- Mesotibia: 1 = black with apex brownish yellow, 2 =- middle part dark, base and apex lighter, 3 = yellow or yellowish white, 4 = pale yellow, middle part dark, 5 = dark with distal half pale

10- Metatibia: 1 = dark brown or black, 2 = dark with apex pale, 3 = black or brown with base white, 4 = pale yellow, often with middle part darker

11- Metasoma: 1 = T1 or T1&T2 yellow, 2 = T1 or T1&T2 and apex pale, 3 = T1-T3 yellow, 4 = dark, black, 5 = female yellow with brown dorsum, except T1&T2, male dark with pale base, 6 = female yellow with brown dorsum except T1&T2, male base and apex pale

12- Ovipositor / mesotibia: 1 = less than 1.2, 2 = 1.3–1.5, 3 = more than 1.5

Table 3. List of anatomical terms and links to URI locations in the Hymenoptera Anatomy Ontology portal.

Term	Definition	URI
antenna	The anatomical cluster that is composed of the scape, pedicel and flagellum.	http://purl.obolibrary.org/obo/HAO_0000101
apical denticle	The spur that is located distally on the gonossiculus.	http://purl.obolibrary.org/obo/HAO_0001574
base	The tergum that is located on abdominal segment 2 AND The tergum that is located on the abdominal segment 3.	http://purl.obolibrary.org/obo/HAO_0000053 and http://purl.obolibrary.org/obo/HAO_0000056
body	The anatomical cluster that is composed of the whole organism but which excludes the antennae, legs and wings.	http://purl.obolibrary.org/obo/HAO_0000182
club	The anatomical cluster composed of the apical flagellomeres that are differentiated by size from the basal flagellomeres.	http://purl.obolibrary.org/obo/HAO_0001185
compound eye	The compound organ that is composed of ommatidia.	http://purl.obolibrary.org/obo/HAO_0000217
costal cell	The membranous region of the forewing anterior to the submarginal vein, measured from the basal constriction that delimits the apex of the humeral plate of the wing to the point at which the submarginal vein touches the leading edge of the wing.	http://purl.obolibrary.org/obo/HAO_0000226
coxa	The leg segment that is connected to the body and to the trochanter via conjunctivae and muscles.	http://purl.obolibrary.org/obo/HAO_0000228
digitus	The sclerite that is located distally on the parossiculus.	http://purl.obolibrary.org/obo/HAO_0000385
edge	The margin that extends along the border of two areas that are oriented differently.	http://purl.obolibrary.org/obo/HAO_0000285
eye margin	The margin of the compound eye.	http://purl.obolibrary.org/obo/HAO_0000672
F1	The flagellomere that is proximally attached to the pedicel.	http://purl.obolibrary.org/obo/HAO_0001148
F2	The flagellomere that is located distal to the first flagellomere.	http://purl.obolibrary.org/obo/HAO_0001883
F3	The flagellomere that is located immediately distal to the second flagellomere.	http://purl.obolibrary.org/obo/HAO_0001895
femur	The leg segment that is distal to the trochanter and proximal to the tibia.	http://purl.obolibrary.org/obo/HAO_0000327
fore wing	The wing that is located on the mesothorax.	http://purl.obolibrary.org/obo/HAO_0000351
frontovertex	The anatomical cluster that is composed of the vertex and the dorsal area of the upper face dorsal to the frontofacial ridge.	http://purl.obolibrary.org/obo/HAO_0001823
genitalia	The anatomical cluster that is composed of the cupula, gonostyle, volsella and the aedeagus.	http://purl.obolibrary.org/obo/HAO_0000312
head	The tagma that is located anterior to the thorax.	http://purl.obolibrary.org/obo/HAO_0000397
hind wing	The wing that is located on the metathorax.	http://purl.obolibrary.org/obo/HAO_0000400
leg	The anatomical cluster that is composed of the coxa and all distal leg segments and is connected to the pectus.	http://purl.obolibrary.org/obo/HAO_0000494

Term	Definition	URI
longitudinal sensillum	The multiporous plate sensillum that is elongate.	http://purl.obolibrary.org/obo/HAO_0001936
mandible	The sclerite that is connected to the cranium along the anterior margin of the oral foramen via the anterior and posterior cranio-mandibular articulations.	http://purl.obolibrary.org/obo/HAO_0000506
margin	The line that delimits the periphery of an area.	http://purl.obolibrary.org/obo/HAO_0000510
marginal vein	The abscissa that is located along the anterior margin of the fore wing and is thought to correspond to the anterior abscissa of the radius (R1).	http://purl.obolibrary.org/obo/HAO_0000512
mesobasitarsus	The basitarsus that is located in the mid leg.	http://purl.obolibrary.org/obo/HAO_0001133
mesocoxa	The coxa that is located on the mid leg.	http://purl.obolibrary.org/obo/HAO_0000635
mesofemur	The femur that is located on the mid leg.	http://purl.obolibrary.org/obo/HAO_0001131
mesoscutum	The area that is located anterior to the transscutal articulation.	http://purl.obolibrary.org/obo/HAO_0001490
mesosoma	The anatomical cluster that is composed of the prothorax, mesothorax and the metapectal-propodeal complex.	http://purl.obolibrary.org/obo/HAO_0000576
mesotibia	The tibia that is located on the mid leg.	http://purl.obolibrary.org/obo/HAO_0001351
mesotibial spur	The tibial spur that is located on the mesotibia.	http://purl.obolibrary.org/obo/HAO_0001120
metabasitarsus	The basitarsus that is located on the hind leg.	http://purl.obolibrary.org/obo/HAO_0001142
metabasitarsus	The basitarsus that is located on the hind leg.	http://purl.obolibrary.org/obo/HAO_0001142
metacoxa	The coxa that is located on the hind leg.	http://purl.obolibrary.org/obo/HAO_0000587
metasoma	The tagma that is connected anteriorly to the metapectal-propodeal complex at the propodeal foramen and consists of abdominal segments.	http://purl.obolibrary.org/obo/HAO_0000626
metatibia	The tibia that is located on the hind leg.	http://purl.obolibrary.org/obo/HAO_0000631
metatibial spur	The tibial spur that is located on the metatibia.	http://purl.obolibrary.org/obo/HAO_0001121
mid lobe of mesoscutum	The area that is located between the notauli.	http://purl.obolibrary.org/obo/HAO_0000520
occipital margin	The edge that separates the occiput from the vertex.	http://purl.obolibrary.org/obo/HAO_0001963
ocellus	The multi-tissue structure that is located on the top of the head, composed of the corneal lens, pigment cell, rhabdoms and synaptic plexus.	http://purl.obolibrary.org/obo/HAO_0000661
ovipositor	The anatomical cluster that is composed of the first valvulae, second valvulae, third valvulae, first valvifers and second valvifers .	http://purl.obolibrary.org/obo/HAO_0000679
pedicel	The antennal segment that is the second segment of the antenna and is connected proximally with the scape and distally with the flagellum.	http://purl.obolibrary.org/obo/HAO_0000706

Term	Definition	URI
phallobase	The anatomical cluster that is composed of the cupulae, gonostipites and volsellae.	http://purl.obolibrary.org/obo/HAO_0000713
posterior ocellus	The ocellus that is paired.	http://purl.obolibrary.org/obo/HAO_0000481
procoxa	The coxa that is located on the fore leg.	http://purl.obolibrary.org/obo/HAO_0001122
profemur	The femur that is located on the fore leg.	http://purl.obolibrary.org/obo/HAO_0001124
protibia	The tibia that is located on the fore leg.	http://purl.obolibrary.org/obo/HAO_0000350
row	The anatomical cluster that is composed of repeated units of anatomical structures.	http://purl.obolibrary.org/obo/HAO_0000901
scape	The antennal segment that is proximal to the pedicel and is connected with the head via the radicle.	http://purl.obolibrary.org/obo/HAO_0000908
sculpture	The area that is located on the sclerite and that is composed of repetitive anatomical structures.	http://purl.obolibrary.org/obo/HAO_0000913
scutellar sensillum	The campaniform sensillum that is paired and is located submedially on the mesoscutellum.	http://purl.obolibrary.org/obo/HAO_0001965
scutellum	The area that is located posteriorly of the transscutal line and is composed of the axillae and the mesoscutellum.	http://purl.obolibrary.org/obo/HAO_0000572
secretory pore	The anatomical space that corresponds to the distal end of an exocrine gland.	http://purl.obolibrary.org/obo/HAO_0001966
seta	The sensillum that is multicellular and consists of trichogen, tormogen, and sense cells.	http://purl.obolibrary.org/obo/HAO_0000935
side lobe	The area that is located between the notaulus and the parascutal carina.	http://purl.obolibrary.org/obo/HAO_0000466
stigma	The patch on the wing that is sclerotized and is located on the anterior margin of the fore wing.	http://purl.obolibrary.org/obo/HAO_0000957
submarginal vein	Basal-most portion of the forewing vein complex that occurs behind the costal cell; measured from the constriction that delimits the humeral plate to the point at which the vein touches the leading edge of the wing apically.	http://purl.obolibrary.org/obo/HAO_0000972
T1	The tergum that is located on abdominal segment 2.	http://purl.obolibrary.org/obo/HAO_0000053
T2	The tergum that is located on the abdominal segment 3.	http://purl.obolibrary.org/obo/HAO_0000056
tarsus	The leg segment that is apical to the tibia.	http://purl.obolibrary.org/obo/HAO_0000992
third valvula	The sclerite that is located posterior to the second valvifer and is connected to the second valvifer via conjuntiva.	http://purl.obolibrary.org/obo/HAO_0001012
tooth	The projection that is located distally on the mandible.	http://purl.obolibrary.org/obo/HAO_0001019
wing	The wing that is located on the mesothorax.	http://purl.obolibrary.org/obo/HAO_0000351

Results and discussion

Following the work of Zehavi and Rosen (1988), we consider the following traits to be diagnostic for the *A. mali* complex: (1) head and body dark except for parts of the metasoma; (2) metafemur pale, (3) a single complete row of setae proximal to the linea calva of the fore wing, with a few additional setae in the angle between this row and the marginal vein; (4) linea calva open (no setae at its posterior edge); (5) meso- and metacoxae dark; (6) metatibia more or less dark. The *A. mali* complex consists of eleven described species, and there are six similar species with either a dark metafemur or more than one line of setae proximal to the linea calva (Table 1). Species within the complex have been distinguished by color and shape of antennal segments (particularly the third funicular segment), color of legs and metasoma, and relative length of ovipositor versus mesotibia (Ashmead 1888; Evans et al. 1995; Gahan 1924; Girault 1913; Haldeman 1851; Hayat 1998; Prinsloo and Neser 1994; Timberlake 1924; Yasnosh 1963; Zehavi and Rosen 1988).

Key to species in the *Aphelinus mali* complex

1 Female: procoxa white or yellowish-white, male: procoxa yellowish-white or grey, both sexes: meso- and metacoxae dark **2**

– All coxae dark in both sexes **4**

2(1) F3 more than twice as long as broad in male, subquadrate in female.... ***engaeus***

– F3 less than twice as long as broad in male and from subquadrate to more than 1.4× as long as broad in female. **3**

3(2) Procoxa yellowish white in male; metatibia yellowish white; metabasitarsus pale; club light brown in male; metasoma dark with base yellow in female and slightly pale in male; ovipositor less than 1.2× middle tibia ..... ***ficusae***

– Procoxa grey in male, particularly on anterior surface; metatibia dark in center; metabasitarsus greyish brown; club yellow in male; metasoma dark with T1&2, venter, and apex yellow in female and base and apex yellow in male; ovipositor more than 1.2× mesotibia..... *glycinis* sp. n.

4(1) F3 longer than broad in male and subquadrate to longer than broad in female; metasoma dark or dark with pale base, but with apex dark **5**

– F3 subquadrate in both male and female; metasoma dark with pale base or pale base and apex..... **8**

5(4) Pro- and metafemur dark; protibia dark or dark with yellow apex; metasoma dark or dark with pale base **6**

– Pro- and metafemur partly yellow; protibia yellow; metasoma dark with pale base..... **7**

6(5) Scape yellow to pale brown with apical third yellow; pedicel and club infuscate brown; metabasitarsus dark; F3 subquadrate in female and 1.2–2× as long as broad in male; metasoma dark with pale base ..... ***basilicus***

– Scape dark brown to black; pedicel yellow in female and yellow to dusky in male; club yellow; metabasitarsus yellow; F3 longer than broad in female and more than 2× as long as broad in male; metasoma dark. ..... ***sanborniae***

7(6) Pro- and mesofemur dark with base and apex pale; protibia dark with pale base and apex, metatibia dark with pale apex; F3 1.2–1.5 as long as broad in female and 1.2–2× as long as broad in males; ovipositor equal to metatibia; metasoma dark with T1–T3 yellow ..... ***paramali***

– Pro- and mesofemur dark with apex pale; protibia dark with brownish yellow apex, metatibia dark; F3 subquadrate in female and 1.2–2× as long as broad in male; ovipositor more than 1.5× mesotibia; metasoma dark with T1 or T1&2 yellow. ..... ***mali***

8(4) Profemur pale yellow, mesofemur pale yellow in female, dark brown in male; metabasitarsus yellow; metasoma dark with base yellow ..... ***spiraecolae***

– Profemur dark with apex pale yellow, mesofemur dark in both sexes; metabasitarsus dark; metasoma dark with base and apex yellow. **9**

9(8) Club more than 2× as long as broad in female; metatibia dark with pale base... **11**

– Club 2× as long as broad in females; metatibia all dark. **10**

10(9) Club and pedicel light brown in female and darker in males; mesofemur dark; mesotibia dark with pale base and apex ..... ***campestris***

– Club pale yellow in female and male; pedicel dusky yellow in female and light brown in male; mesotibia dark with brownish yellow apex ..... ***gossypii***

11(9) Club more than 3× as long as broad in males; scape yellowish white in female and infuscate brown in male; mesotibia dark with base and apex pale ..... ***rhamni* sp. n.**

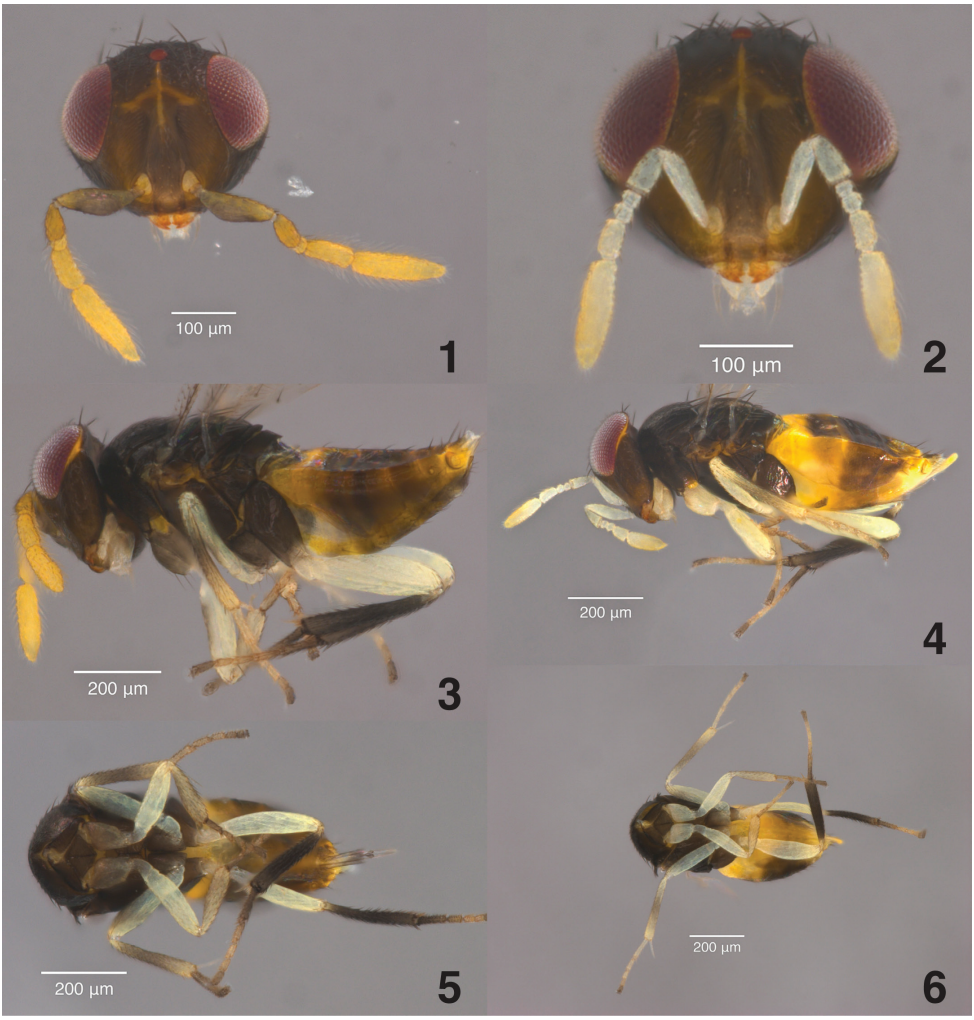
– Club less than 3× as long as broad in males; scape dark brown with distal half yellow in both sexes; mesotibia dark with distal half yellow ..... ***coreae* sp. n.**

Taxonomy

***Aphelinus glycinis* Hopper & Woolley, sp. n.**
urn:lsid:zoobank.org:act:1132B1E4-8F2E-4FF3-9E6B-30FAFD497EA1
http://species-id.net/wiki/Aphelinus_glycinis

Figs 1–14

Diagnosis. Female. Head and mesosoma dark brown to black; legs with procoxa yellowish white, meso- and metacoxae dark brown to black, femora yellowish white, protibia yellowish white, mesotibia yellowish white with center greyish, metatibia dark grey to black with base pale; metasoma with base, apex, and venter yellow, remainder brown; antenna white to yellowish white; F3 1.3–1.7 times as long as broad; club 3.2 times as long as broad. **Male** similar except procoxa grey; pro- and mesofemur sometimes with darkened center; metasoma brown with base and apex yellow; scape dark greyish brown with greyish yellow distal tip, swollen in center, maximum width 3× distal end width, with three to five



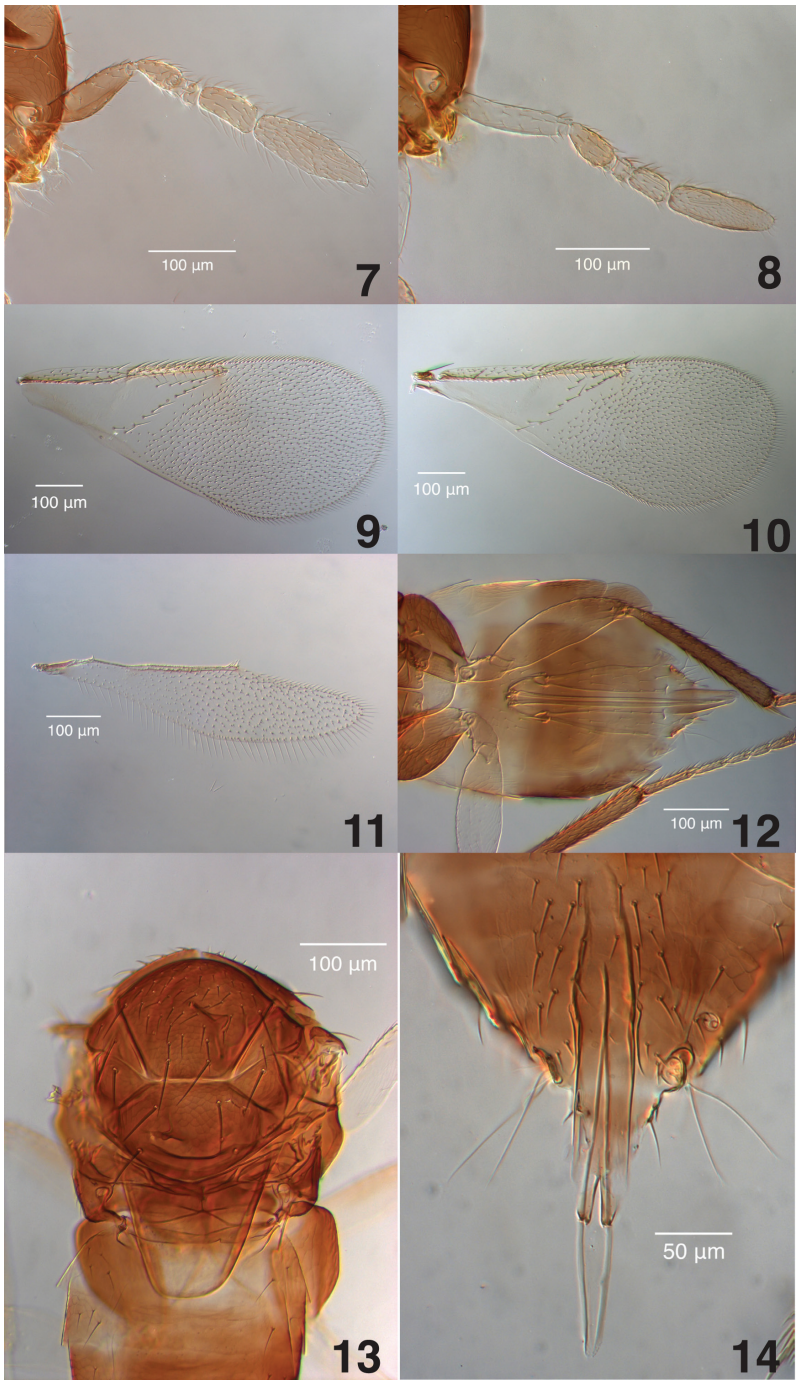
Figures 1–6. *Aphelinus glycinis* sp. n. paratype specimens in 95% ethanol. **1** male antennae and face **2** female antennae and face **3** male, lateral view **4** female, lateral view **5** male, ventral view **6** female, ventral view.

volcano-shaped secretory pores in a single line on ventral surface, pedicel greyish yellow, third funicle more than 2 times as long as broad, club 3.9 times as long as broad.

Description. Female (Figs 2, 4, 6, 8, 10, 11, 12, 13).

Body length. 0.77–0.93 (Holotype 0.90 mm).

Head. (Figs 2, 8) Head 1.3× as broad as high in frontal view, about as broad as mesosoma; frontovertex width 0.4× of head width, 1.2× as long as broad, and 0.8× as broad as scape length; posterior ocelli 1.0× their diameter from eye margin, 3× their diameter from one another, and 0.33× their diameter from occipital margin; mandible with 2 acute teeth and a broad truncate surface below the teeth, antenna as in Fig. 8 with scape 4.8× as long as broad, pedicel 2.2× as long as broad, F1 anneliform, 1.7× as



Figures 7–14. *Aphelinus glycinis* sp. n., slide-mounted paratypes. **7** male antenna (TAMU x0616203) **8** female antenna (TAMU x0616201) **9** male fore wing (TAMU x0616206) **10** female fore wing (TAMU x0616201) **11** female hind wing (TAMU x0616204) **12** female metasoma (TAMU x0616204) **13** female mesosoma (TAMU x0616211) **14** male genitalia (TAMU x0616206).

wide as long, F2 1.2× as broad as long, F3 1.6–1.7× as long as broad, club 3.2× as long as broad, 2.7× times as long as F3, and with 6–7 longitudinal sensilla.

Mesosoma. (Figs 4, 6, 13) Mesoscutum and scutellum with fine reticulate sculpture, longest diameter of reticulations approximately twice the diameter of the scutellar sensilla, interior of reticulations with fine, granulate surface (visible only in slide-mounts under high magnification); mid-lobe of mesoscutum with 2 pairs of long setae and about 40 short setae, side lobes each with 2 long and 1 short setae; scutellum with 2 pairs of long setae and two sensilla directly posterior to the anterior pair of setae, scutellar sensilla slightly posterior to middle of scutellum; mesotibial spur 0.8× mesobasitarsus; metatibial spur 0.6× metabasitarsus.

Fore wing. (Fig. 10) 2.3× as long as broad; costal cell with 1 complete row of dorsal setae and 2 rows of ventral setae, the posterior row extending from under the proximal end of the marginal vein almost to stigma, costal cell 1.2× longer than marginal vein; submarginal vein with 5–6 setae; marginal vein with 10 setae along the margin; stigmal vein short with stigma rounded; delta region proximal to linea calva with one complete line of 13–15 setae and 2–6 additional setae in angle with marginal vein, linea calva with no dorsal setae at its posterior edge; wing distal to linea calva with dense, evenly spaced, dorsal setae and much smaller ventral setae.

Hind wing. (Fig. 11) 4.3× as long as broad, marginal fringe 0.26× wing width.

Metasoma. (Figs 4, 6, 12) 1.2× as long as mesosoma; ovipositor inserted at middle of metasoma, slightly exerted distally, 1.3× as long as meso- and metatibiae; third valvula one-third length of ovipositor.

Color. (Figs 2, 4, 6) Head and mesosoma dark brown to black; legs with procoxa yellowish white, meso- and metacoxae dark brown to black, femora yellowish white, protibia yellowish white, mesotibia yellowish white with center greyish, metatibia dark grey to black base pale, tarsi pale brown with tips and metabasitarsus greyish brown; metasoma with base (T1 and T2), apex, and venter yellow, remainder brown; antennae yellowish white; compound eyes burgundy, and ocelli red in life, both silver-colored in dried specimens.

Male (Figs 1, 3, 5, 7, 9, 14). Similar to female except:

Head. (Figs 1, 7) Antenna with scape swollen in middle, 3.4 as long as broad, maximum width 3× distal end width, with 3–5 volcano-shaped secretory pores in line on ventral surface, pedicel 2.1× as long as broad, F1 anneliform, 1.8× as wide as long, F2 1.5× as broad as long, F3 2.0× as long as broad, club 3.9× as long as broad, 2.1× as long as F3, and with 4–6 longitudinal sensilla.

Metasoma. (Figs 3, 5, 14) About the same length as mesosoma, phallobase of genitalia including digiti 4.5× longer than broad, digiti about twice longer than broad and with two apical denticles.

Color. (Figs 1, 3, 5) Legs with procoxa grey, pro- and mesofemora sometimes with darkened centers; metasoma brown with base and apex yellow; scape dark greyish brown with greyish yellow distal tip, pedicel greyish yellow.

Holotype female (card-mounted, deposited in USNM, USNM ENT 00703637). "China: Liaoning, Xiuyan | 40°18'N, 123°14'E | 11.vii.2007, K. Hoelmer || ex: *Aphis*

glycines | on: soybean | plots 1/3, 2007/007 || From Lab Culture | USDA-ARS-BIIRU | Newark, Delaware"

Paratypes (USNM, TAMU, BMNH)**.** 30 card-mounted and 4 slide-mounted ♀♀, 14 card-mounted and 4 slide-mounted ♂♂ with same data as holotype. 13 card-mounted and 4 slide-mounted ♀♀ and 9 card-mounted and 3 slide-mounted females: **China**, Liaoning, Xiuyan, 40˚20'N 116˚6'E, 12.vii.2007, K. Hoelmer, ex: *Aphis glycines* on: soybean, plot 2, 2008/008, from Lab Culture, USDA-ARS-BIIRU, Newark, Delaware, all bearing TAMU accession numbers.

Hosts. In the field, *Aphis glycines* is the only known host. In laboratory experiments, *A. glycinis* parasitizes *A. glycines* and closely related species in the genus *Aphis*.

Etymology. This species is named for the host from which it was collected. The species epithet is a noun in genitive case.

Relationships. *Aphelinus glycinis* is closest to *A. engaeus* and *A. ficusae* Prinsloo and Neser based on our matrix of traits (Table 2). *Aphelinus glycinis* differs from *A. engaeus* in having elongated third funicle segments in males and females, and it differs from *A. ficusae* in having an ovipositor more than 1.2× as long as the mesotibia and grey procoxa in males. It also differs from these species in its aphid hosts and geographical distribution. *Aphelinus glycinis* is a specialist on *Aphis* species close to *Aphis glycines*, but *A. engaeus* is reported from *Schizaphis graminum* (Rondani) and *Sitobion ochnearum* (Eastop) and *A. ficusae* was reared from an undetermined aphid on *Ficus sycomorus* (Prinsloo and Neser 1994). Furthermore, *Aphelinus glycinis* was collected in northeastern China, whereas *A. engaeus* and *A. ficusae* have been reported only from South Africa.

Aphelinus rhamni Hopper & Woolley, sp. n.

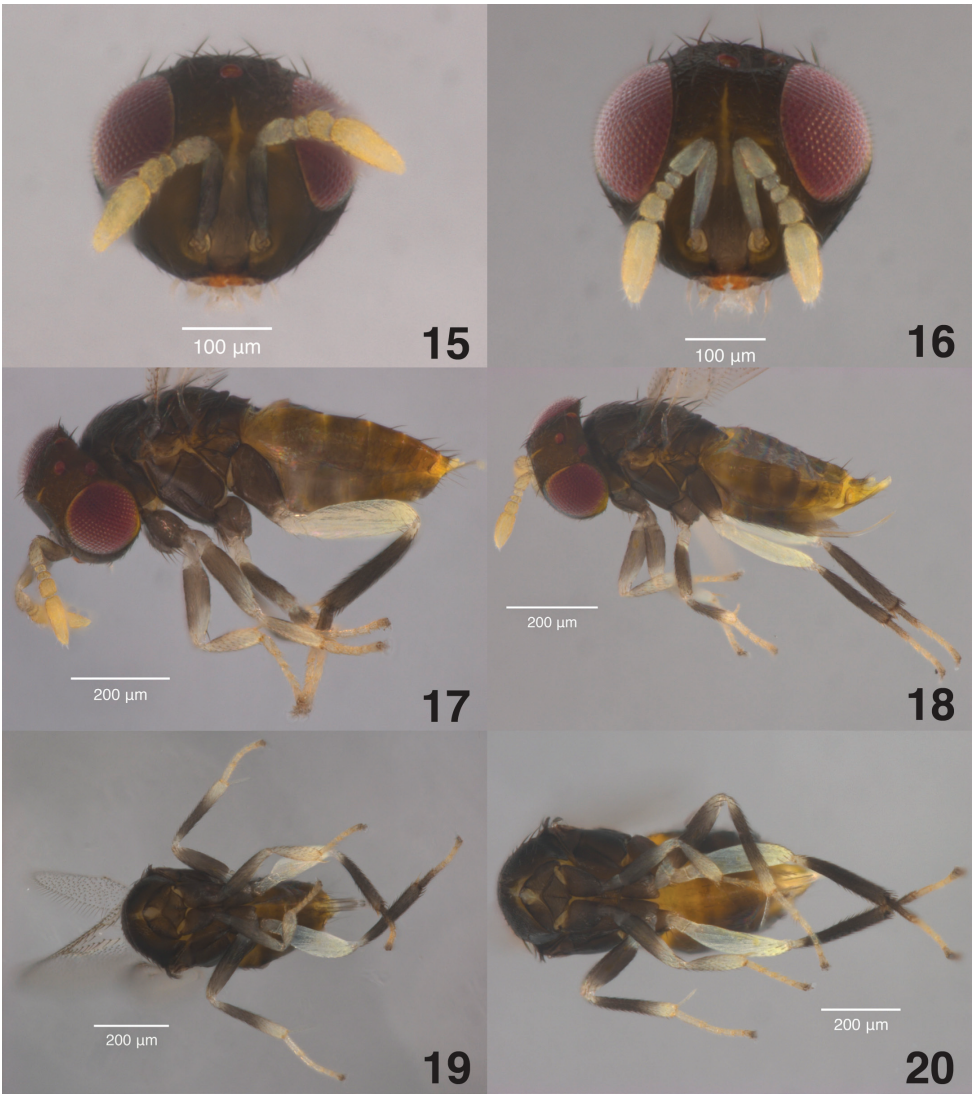
urn:lsid:zoobank.org:act:1132B1E4-8F2E-4FF3-9E6B-30FAFD497EA1
http://species-id.net/Aphelinus_rhamni
Figs 15–28

Diagnosis. Females. Head and mesosoma dark brown to black; legs with coxae dark brown to black, profemur dark grey with pale apex, mesofemur dark grey to black, metafemur white, protibia white with pale greyish base, mesotibia dark grey to black with pale base and apex, and metatibia dark grey to black with pale base; metasoma yellowish brown with base and apex yellow; antennae yellow with basal half of scape and pedicel sometimes greyish; F3 quadrate; club 2.8 times as long as broad. **Males** similar except scape swollen in middle, 3× broader in middle than at distal end, with 2 or 3 volcano-shaped secretory pores; scape dark yellowish grey, pedicel pale greyish yellow; club 3.3 times as long as broad.

Description. Female (Figs 16, 18, 20, 22, 24, 25, 26, 27).

Body length. 0.75–0.94 (Holotype 0.87 mm).

Head. (Figs 16, 22) Head 1.2× as broad as high in frontal view, about as broad as mesosoma; frontovertex 0.4× head width and as broad as scape length; posterior ocelli



Figures 15–20. *Aphelinus rhamni* sp. n., paratype specimens in 95% ethanol. **15** male antennae and face **16** female antennae and face **17** male, lateral view **18** female, lateral view **19** male, ventral view **20** female, ventral view.

0.5× their diameter from eye margin, 3.0× their diameter from one another, and 0.33× their diameter from occipital margin; mandible with 2 acute teeth and a broad truncate surface below the teeth; antennae as in Fig. 21 with scape 4.8 longer than broad, pedicel 1.8× as long as broad, F1 anneliform, F2 1.5× as broad as long, F3 quadrate, club 2.8× as long as broad and 3.3× times longer than F3, with 4–6 longitudinal sensilla.

Mesosoma. (Figs 18, 20, 27) Mesoscutum and scutellum with fine reticulate sculpture, longest diameter of reticulations approximately twice the diameter of scutellar

Figures 21–28. *Aphelinus rhamni* sp. n., slide-mounted paratypes. **21** male antenna (TAMU x0616221) **22** female antenna (TAMU x0616215) **23** male fore wing (TAMU x0616217) **24** female fore wing (TAMU x0616215) **25** female hind wing (TAMU x0616129) **26** female metasoma (TAMU x0616214) **27** female mesosoma (TAMU x0616129) **28** male genitalia (TAMU x0616217).

sensilla, interior of reticulations with fine, granulate surface (visible only in slide-mounts under high magnification); mid-lobe of mesoscutum with 2 pairs of long setae and 35–40 short setae, side lobes each with 2 long and 1 short seta; scutellum with 2 pairs of long setae, pair of scutellar sensilla directly posterior to the anterior pair of setae and slightly posterior to middle of scutellum; mesotibial spur equal in length to mesobasitarsus; metatibial spur 0.5× metabasitarsus.

Fore wing. (Fig. 24) 2.2× as long as broad ; costal cell with 1 row of dorsal setae and two rows of ventral setae, the posterior row extending from under the proximal end of the marginal vein almost to stigma, costal cell 1.1× as long as marginal vein; submarginal vein with 5 setae, marginal vein with 10 setae along the anterior margin; stigmal vein short with stigma rounded; delta region proximal to linea calva with one complete line of 13–15 setae and 2–6 additional setae in angle with marginal vein, linea calva with no setae at its posterior edge; wing distal to linea calva with evenly spaced, dense dorsal setae and much smaller ventral setae.

Hind wing. (Fig. 25) 3.9× longer than broad, marginal fringe 0.23× wing width.

Metasoma. (Figs 18, 20, 26) 1.4× as long as mesosoma; ovipositor inserted at middle of metasoma, slightly exerted distally, 1.3× longer than metatibia and mesotibia; third valvulae one-third the length of ovipositor.

Color. (Figs 16, 18, 20) Head and mesosoma dark brown to black; legs with coxae dark brown to black, profemur dark grey with pale apex, mesofemur dark grey to black, metafemur white, protibia white with pale greyish base, mesotibia dark grey to black with pale base and apex, and metatibia dark grey to black with pale base; metasoma yellowish brown with venter of T1 and apex yellow; antennae yellow sometimes with basal half of scape and pedicel greyish yellow; compound eyes dark burgundy and ocelli red in life, both silver-colored in dried specimens.

Male (Figs 15, 17, 19, 21, 23, 28). Similar to female except:

Body length. 0.66–0.78 mm.

Head. (Figs 15, 21) Antenna with scape swollen in center, 3.1× as long as broad, maximum width 3× width at distal end, with 2–3 volcano-shaped secretory pores in single line on ventral surface, pedicel 1.8× as long as broad, F1 subquadrate, 1.1× as broad as long, F2 shorter, 1.4× as broad as long, F3 trapezoidal, 1.1× longer than width at apex, 1.5× as long as wide at base, club 3.3× as long as broad, with 4 longitudinal sensilla.

Metasoma. 0.7× length of mesoma

Color. (Figs 15, 17, 19) Scape dark yellowish grey, pedicel pale greyish yellow, base of metasoma pale brown and with yellow region at apex smaller.

Holotype female (card-mounted, deposited in USNM, USNM ENT 00763638)**.** "China, Daxing (Beijing) | 39˚48'N, 116˚28'E | 10.ix.2005, K. Hoelmer || ex: *Aphis glycines* | on: *Rhamnus* sp. | 2005/005 || From Lab Culture | USDA-ARS-BIIRU | Newark, Delaware"

Paratypes (USNM, TAMU, BMNH)**.** 33 card-mounted and 6 slide-mounted females, 19 card-mounted and 3 slide-mounted males with same data as holotype. 9 card-mounted and 2 slide-mounted females, 6 card-mounted and 2 slide-mounted males: **China**: Daxing (Beijing), 39˚48'N 116˚28'E, 10.iv.2004, W. Meikle coll.,

ex: *Aphis glycines* on *Rhamnus* sp., 2004/008, from lab culture, USDA-ARS-BIIRU, Newark, Delaware.

Host. In the field, *Aphis glycines* is the only known host. In laboratory experiments, *A. rhamni* parasitizes *A. glycines* and closely related species in the genus *Aphis*, and rarely *Rhopalosiphum padi* L. and *Schizaphis graminum*.

Etymology. This species is named for the primary host plant of the aphid species from which it was collected. The species epithet is a noun in genitive case.

Relationships. *Aphelinus campestris* and *Aphelinus gossypii* are the closest described species to *A. rhamni* based on our matrix of traits (Table 2). *Aphelinus rhamni* differs from both species in having a more elongate club and in coloration of the metatibia. *Aphelinus rhamni* has a much narrower host range than *A. gossypii*, which is reported from at least 18 species of aphids in 10 genera and two tribes, including species which *A. rhamni* does not parasitize in laboratory experiments.

Aphelinus coreae sp. n.

urn:lsid:zoobank.org:act:F4B3A880-2136-474C-815C-13406F2A48A0
http://species-id.net/Aphelinus_coreae
Figs 29–42

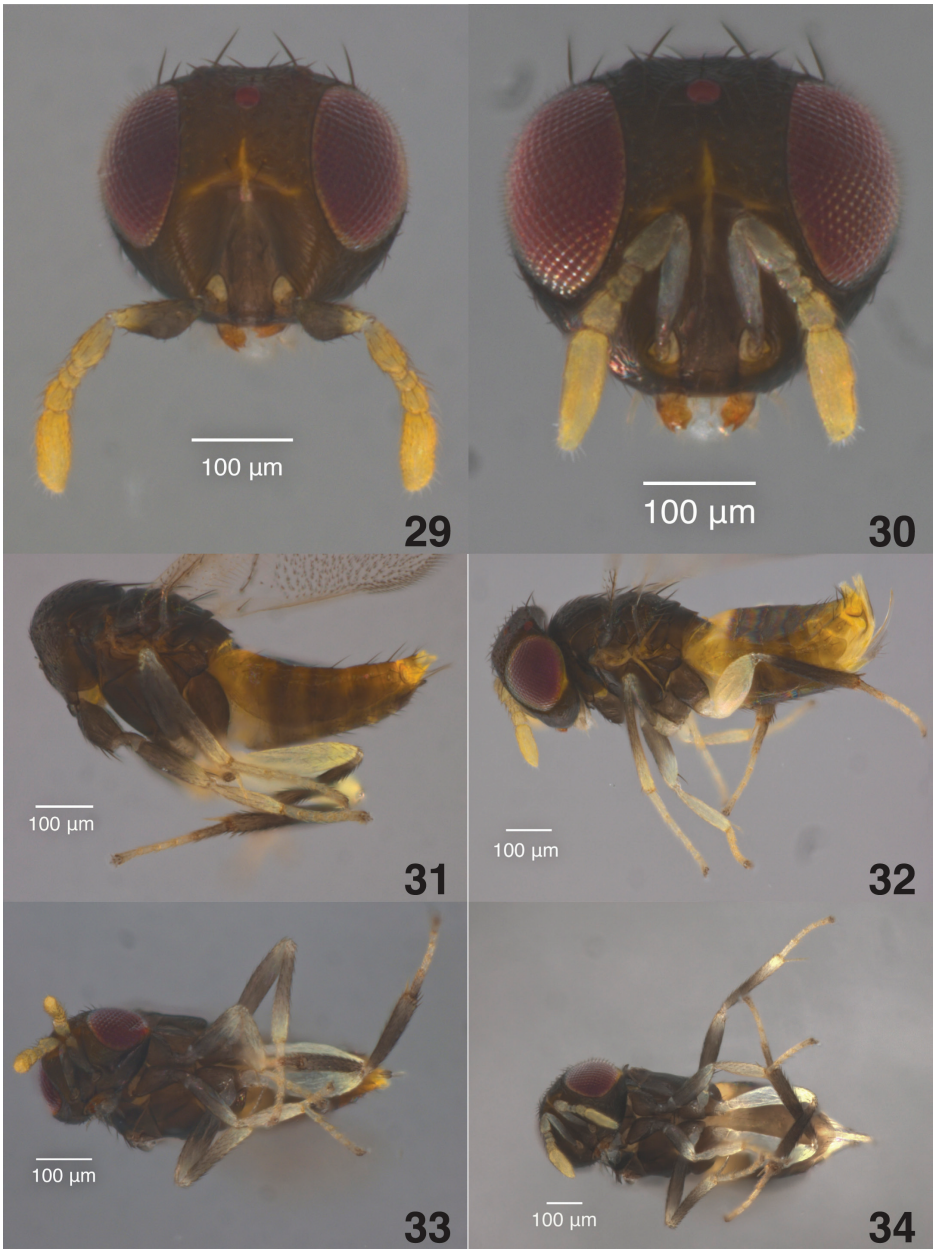
Diagnosis. Females. Head and thorax dark brown to black; legs with coxae dark brown to black, profemur dark grey with distal half pale, mesofemur dark grey to black, metafemur pale yellowish white, protibia pale yellowish white to somewhat fuscous, mesotibia dark grey to black with distal half pale, and metatibia dark grey to black with pale base; metasoma dark brown with base and apex yellow; antennae yellow; F3 quadrate. **Males** similar except scape swollen in middle, 2.0× as broad in middle than at distal end, with two or occasionally three circular secretory pores in the middle of a shallow depression on ventral surface, scape dark yellowish grey with distal half yellow, pedicel greyish yellow.

Description. Female (Figs 30, 32, 34, 36, 38, 39, 40, 41).

Body length. 0.80–0.93 (Holotype 0.93 mm).

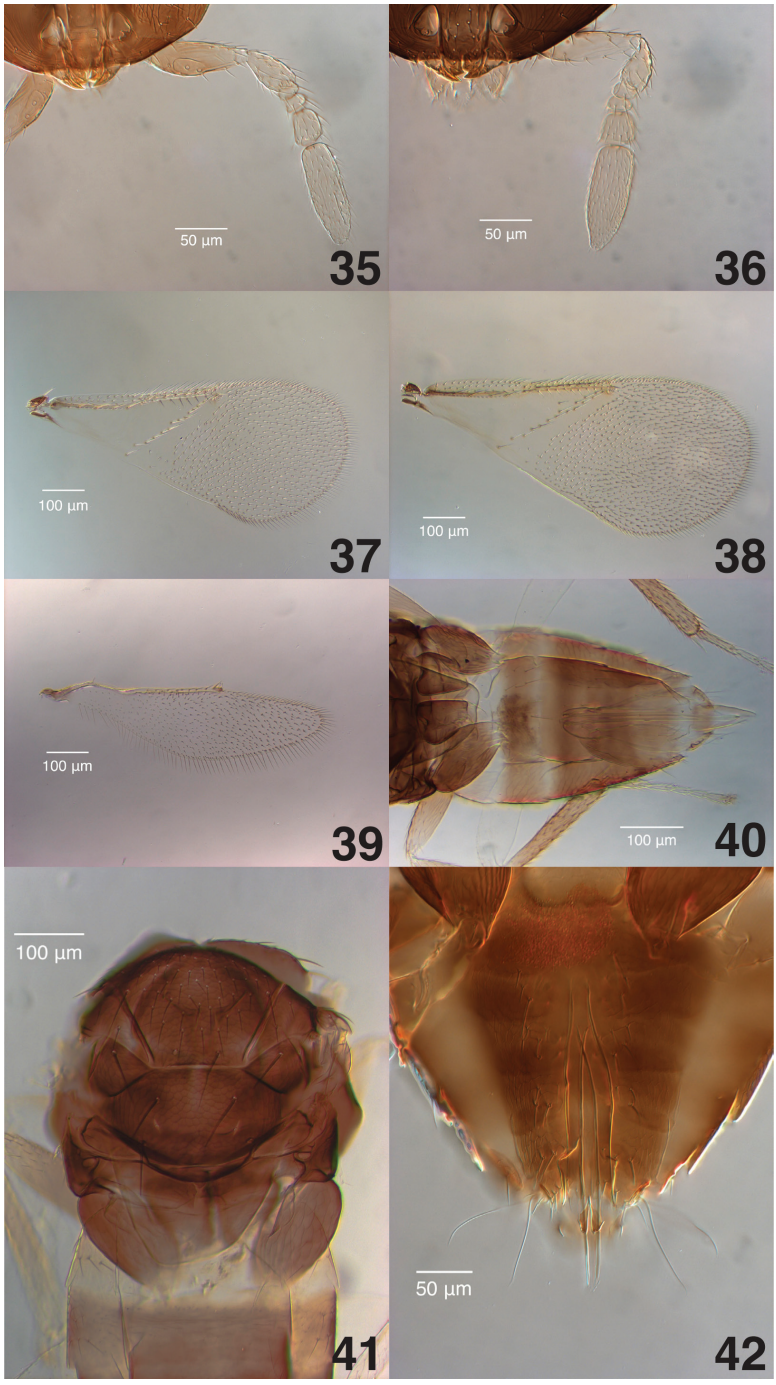
Head. (Figs 30, 36) Head 1.3× as broad as high in frontal view, about as broad as mesosoma; frontovertex 0.4× head width and as broad as scape length; posterior ocelli approximately their own diameter from eye margin, 5× their diameter from one another, and 0.5× their diameter from occipital margin; mandible with two acute teeth and a broad truncate surface below the teeth, ventral tooth sometimes not distinct; antennae as in Figs 30 and 36 with scape 4.0× as long as broad, pedicel 1.6× as long as broad, F1 anneliform, F2 1.4× as broad as long, F3 subquadrate or very slightly broader than long, club 3.75× as long as broad and 3.5× longer than F3, with 7–8 linear sensilla.

Mesosoma. (Figs 32, 34, 41) Mesosoma and scutellum with fine reticulate sculpture, longest diameter of reticulations approximately 2–3× diameter of scutellar sensilla; interior of reticulations with fine, granulate surface (visible only in slide-mounts under high magnification), mid-lobe of mesoscutum with 2 pairs of long setae and about 40–60



Figures 29–34. *Aphelinus coreae* sp. n., paratype specimens in 95% ethanol. **29** male antennae and face **30** female antennae and face **31** male, lateral view **32** female, lateral view **33** male, ventral view **34** female, ventral view.

short setae, side lobes each with 2 long and 1–2 short setae; scutellum with 2 pairs of long setae; pair of scutellar sensilla approximately equidistant from anterior and posterior pairs of long setae; mesotibial spur 1.1× mesobasitarsus; metatibial spur 0.6× metabasitarsus.



Figures 35–42. *Aphelinus coreae* sp. n., slide-mounted paratypes. **35** male antenna (TAMU x0616221) **36** female antenna (TAMU x0616215) **37** male fore wing (TAMU x0616217) **38** female fore wing (TAMU x0616215) **39** female hind wing (TAMU x0616129) **40** female metasoma (TAMU x0616214) **41** female mesosoma (TAMU x0616129) **42** male genitalia (TAMU x0616217).

Fore wing. (Fig. 38) 2.2× as long as broad; costal cell with 1 row of dorsal setae and 2 rows of ventral setae, the posterior row extending from under the distal end of the submarginal vein almost to stigma, costal cell 1.3× as long as marginal vein; submarginal vein with 5 setae; marginal vein with 12 setae along the anterior margin; stigmal vein short with stigma rounded; delta region proximal to linea calva with one complete line of 12–13 setae and 2–5 additional setae in angle with marginal vein, linea calva with no setae at its posterior edge; wing distal to linea calva with evenly spaced, dense dorsal setae and much smaller ventral setae.

Hind wing. (Fig. 39) 3.9× as long as broad, marginal fringe 0.26× wing width.

Metasoma. (Figs 32, 34, 40) 1.1× as long as mesosoma; ovipositor inserted at basal third of metasoma, slightly exerted distally, 1.4× as long as metatibia or mesotibia; third valvula 0.28× length of ovipositor.

Color. (Figs 30, 32, 34) Head and mesosoma dark brown to black; legs with coxae dark brown to black, profemur dark grey with distal half pale, mesofemur dark grey to black, metafemur pale yellowish white, protibia pale yellowish white to somewhat fuscous, mesotibia dark grey to black with distal half pale, and metatibia dark grey to black with pale base; metasoma dark brown with base and apex yellow; antennae yellow; compound eyes dark burgundy, ocelli red in life, both silver-colored in dried specimens.

Male (Figs 29, 31, 33, 35, 37, 42). Similar to female except:

Body length. 0.68–0.81 mm.

Head. (Figs 29, 35) Antenna with scape swollen in center, 3.3× as long as broad, maximum width 2× distal width, with 2 or 3 circular, secretory pores in the middle of a shallow depression on ventral surface, pedicel 2.0× longer than broad, F1 and F2 1.4× broader than long, F3 rectangular, 1.3× as long as wide at apex, club 3.2× as long as broad, with 3–4 longitudinal sensilla.

Metasoma. (Figs 31, 33, 42) 1.5× length of mesoma.

Color. (Figs 29, 31, 33) Scape dark yellowish grey with distal half yellow, pedicel greyish yellow.

Holotype female (card-mounted, deposited in USNM, USNM ENTO 00763639)**.** "Korea, Gyeongsangnam Province, Miryang |35˚30'N, 128˚44'E | 11.viii.2009, K. Hoelmer || ex: *Aphis glycines* | on: soybean | 2009/011 || From Lab Culture | USDA-ARS-BIIRU | Newark, Delaware"

Paratypes (USNM, TAMU, BMNH)**.** 13 card-mounted and 3 slide-mounted ♀♀ and 9 card-mounted and 5 slide-mounted ♂♂ with same data as holotype.

Other material examined. None.

Host. In the field, *Aphis glycines* is the only known host. In laboratory experiments, *A. coreae* parasitizes *A. glycines* and other species in the genus *Aphis*, and occasionally *Rhopalosiphum padi* and *Schizaphis graminum*.

Etymology. This species is named for its country of origin. The species epithet is a noun in genitive case.

Relationships. *Aphelinus campestris* and *Aphelinus gossypii* are the closest described species *A. coreae* based on our matrix of traits (Table 2). *Aphelinus coreae* differs from both species in having a more elongate club in females and in coloration of the scape

and mesotibia. Like *A. rhamni*, *A. coreae* has a much narrower host range than *A. gossypii*. *Aphelinus coreae* is very close to *A. rhamni*, but male *A. coreae* have shorter clubs and, as noted in the key, the two species differ in coloration of scape and mesotibia. Although difficult to distinguish, these species are reproductively isolated in laboratory crosses. Their DNA differs by 2130 fixed substitutions and 293 indels across 1.8 megabases of homologous DNA sequence. They also differ in host specificity: *A. coreae* parasitizes species of *Aphis*, e.g. *A. nerii* Boyer de Fonscolombe and *A. rumicis* L., not parasitized by *A. rhamni* in laboratory experiments.

Acknowledgements

Alyssa Mann, undergraduate student at Texas A&M University, helped with digital imaging and preparation of figures. Kathryn Lanier, USDA-ARS, Newark, Delaware, reared the cultures of *A. coreae*, *A. glycinis* and *A. rhamni*. We thank István Miko for his generous help in reading the ms and helping us to standardize our terminology according to the Hymenoptera Anatomy Ontology project, and for providing the table of uri's for anatomical terms used in the paper. We thank the editors and two anonymous reviewers for their suggestions. This research was supported in part by funding from the North Central Soybean Research Program (KRH) and NSF award DEB 0730616 (JBW).

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