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Parasitoids of the invasive cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera, Pseudococcidae), in Iraq

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Abstract *Phenacoccus solenopsis* Tinsley (Hemiptera, Pseudococcidae) is an invasive mealybug globally, having originated in North America. During routine rearing of this pest in a plant nursery in Karbala, Iraq in June 2023, three species of parasitoid wasps (Hymenoptera, Chalcicoidea) emerged from the mealybug colonies, or were extracted as pupae from parasitised mealybugs. They were identified using both morphology and DNA sequencing as *Aenasius arizonensis* (Girault), *Cheiloneurus nankingensis* Li and Zu (Encyrtidae), and *Marietta pictus* (André) (Aphelinidae). The latter two species represent new country records for Iraq. All three species are diagnosed and illustrated below, with summaries of their distributions and biology. The importance of having databases available of barcodes and other diagnostic sequence data is emphasised in the context of biological control programmes.

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Introduction

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera, Pseudococcidae), is a major global pest that has been reported feeding on more than 226 host plant genera belonging to 66 plant families (García Morales et al. 2016). Many of these plant hosts are economically important, such as cotton and several horticultural crops in both indoor and outdoor production (Culik and Gullan 2005; Hodgson et al. 2008; Afzal et al. 2009; Wang et al. 2009; Muniappan 2010; Zhu et al. 2011; Sanaa et al. 2020). Infestation by *P. solenopsis* can cause major yield loss in cotton, reducing the crop yield by up to 40–50% as was reported from India (Jhala et al. 2008). So far, *P. solenopsis* has been reported from 75 countries in various biogeographical zones including Southeast Asia, North Africa, the Mediterranean and China (García Morales et al. 2016; Li et al. 2020). In Iraq, cotton mealybug was reported for the first time in 2014 from Baghdad Province (Abdul-Rassoul et al. 2015).

Fifty species of Hymenoptera have been found associated with *P. solenopsis* (García Morales et al. 2016), of which 18 were recorded recently in a comprehensive study from China (Chen et al. 2021). Ram et al. (2009) indicated that the parasitoid *A. arizonensis* was very active against *P. solenopsis* in India, and observed that parasitoid levels on cotton and other host plants were 37.6% and 47.2% in 2008 and 2009, respectively. The current study was conducted to determine the identity of parasitoids of *P. solenopsis* in Karbala province, Iraq, to quantify parasitoidism levels, and to determine the efficacy of *Aenasius arizonensis* for the classical biological control of *P. solenopsis* currently being undertaken.

Materials and methods

Specimen slide mounting and examination: *Phenacoccus solenopsis*

Samples of mealybug colonies were collected directly into 95% ethanol and transported to the Natural History Museum, London (NHMUK) for identification. DNA was extracted and sequenced using the protocol described below. A total of eight adult female mealybugs were mounted in Canada balsam on standard microscope slides using the protocol described in Sirisena et al. (2013). Specimens were stained with acid fuchsin. Detailed images of species-diagnostic characters were captured on an Olympus BX63 microscope using Nomarski Differential Interference Contrast ("DIC") illumination. These images were used for authoritative identification.

Specimen mounting and examination: parasitoids

Parasitoid samples were collected from a plant nursery in Karbala Governorate, Iraq using traps in July 2023. Specimens were collected directly into 95% alcohol and brought to the Natural History Museum in August 2023. All material was examined on microscope slides for morphological characters using an Olympus BX63 microscope with DIC and 10×, 20×and 40×objectives. Since the lysis buffer used for DNA extraction (see below) contains proteinase K, there may be no need to clear the body with KOH as is usually performed before slide mounting. However, encyrtids often require further clearing, especially of the eye pigment, by immersion in 10% KOH for between 10 and 60 min. Specimens were extracted from the lysis buffer with an adjustable volume pipette (0.5–10 µl) set at 3–4 µl to reduce liquid loss and transferred to distilled water to remove unwanted reagents. They were then dehydrated using a series of ethanol solutions of increasing concentration and cleared in clove oil as described by Noyes (1982). Specimens were dissected, and wings, antennae, head, and body were mounted separately under different coverslips in Canada balsam following Noyes (1982), with the exception that the meso- and meta-somata were not separated. Body colour was observed on both card-mounted specimens, and on slide-mounted specimens in which the body pigmentation remains preserved.

DNA extraction and sequencing

For both *P. solenopsis* and its parasitoids, DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit with modifications as described in Cruaud et al. (2019). Entire specimens were lysed for 8 h without grinding. The primer pairs 28SF: 5′ CCCSSGTAATT TAAGCATATTA 3′ (Whiting et al. 1997) and 28SR: 5′ TCGGAAGGAACCAGCTAC 3 (Whiting et al. 1997; Park and O'Foighil 2000) were used to amplify an approximately 750 bp fragment from the 5′ end of the nuclear ribosomal 28S gene spanning the D2–D3 region. PCR conditions were as described in Fusu and Polaszek (2017). All PCR products were checked by gel electrophoresis in 1% agarose gels, cleaned using AxyPrep PCR clean-up beads as per manufacturer's instructions, then sequenced bi-directionally using BigDye terminator reaction mix v.3.1 in a 3730xl DNA analyser (Applied Biosystems) at the NHMUK sequencing facility.

28S D2-D3, and a proportion of CO1 sequences (details under each species) were edited and analysed using Geneious Prime (version 2023.2.1). Resulting contigs were BLASTed into GenBank. CO1 amplicons for three *Cheiloneurus nankingensis* specimens were obtained by PCR amplification of a 710 bp fragment using the "universal" primer pair LCO1490: 5′-GGTCAACAAATCATAAAGATA TTGG-3′ and HC02198: 5′-TAAACTTCAGGGTGA CCAAAAAATCA-3′ (Folmer et al. 1994; Beasley 2023). Amplicons were prepared for sequencing with the Ligation Sequencing Kit V14 (SQK-LSK114) by Oxford Nanopore Technologies, and sequenced on a GridION using a Flongle (10.4.1). DNA barcodes were demultiplexed, polished and aligned with ONT-Barcoder v2.3.0 (Srivathsan et al. 2021).

Results

Identification of *Phenacoccus solenopsis*

The identity of *P. solenopsis* was confirmed from images of the slide-mounted adult females, using criteria given in Zhao et al. (2014). The specimens were placed in the genus *Phenacoccus* because they have 18 pairs of cerarii, antennae usually nine segmented, each claw with a denticle, lanceolate dorsal setae, and circulus sometimes flaccid and produced laterally. However, they differ from many *Phenacoccus* species in lacking dorsal multilocular disc-pores and ventral quinquelocular pores. *P. solenopsis* differs from *P. solani* Ferris, its closest congener, by having (contrasting character states in *P. solani* given in parentheses): (1) ventral multilocular disc-pores on midline of abdominal segment VII scattered between anterior and posterior margins of segment, or present in rows along both anterior and posterior edges of that segment (disc-pores on midline of segment VII present in a row along posterior edge only), (2) a few translucent pores present on apical end of hind femur (hind femur without translucent pores), and (3) small clusters of ventral multilocular disc pores often present on submargins of some or all of abdominal segments I–VII (venter without submarginal disc-pores).

A 67 bp contig from single forward and reverse sequences of 28S D2-D3 BLASTed had *P. solenopsis* as the top three hits, with pairwise identity at 98.9, 99.3, 99.3%, respectively, and percentage identical sites at 98.5, 99.1, 99.1%, respectively. The sequence has been deposited in GenBank under Accession No. PQ374133.

Material examined: Eight♀♀ Iraq: Karbala 32° 40′ 32.0″ N, 44° 09′ 52.1″ E 15/03/2023 col. AH Khudhair *Phenacoccus solenopsis* GW Watson det. 02/2024 NHMUK DNA1851.

Identification of parasitoids

Three parasitoid species were identified using integrated morphological and molecular data. Their diagnostic morphological characters, distributions and biologies are summarised below. DNA sequence data are discussed, and GenBank accession numbers supplied.

Encyrtidae

Aenasius arizonensis (Girault)

Chalcaspis arizonensis (Girault 1915; p. 280).

Aenasius arizonensis (Girault); Noyes and Woolley (1994; p. 1330).

Aenasius bambawalei (Hayat 2009; p. 25), synonymised by Fallahzadeh et al (2014, p. 516).

Morphological diagnosis: Female (Fig. 1): Body generally squat and robust; funicle 7-segmented, Antennal flagellum entirely expanded, rhomboid, with the apex obliquely truncate. Frontovertex with conspicuous piliferous punctures giving it an appearance similar to that of the surface of a thimble or golf ball, fore wing infuscate, apex of postmarginal and stigmal veins connected by a naked, hyaline streak. Postmarginal vein shorter than stigma vein (Hayat 2009). Male (Fig. 2): Generally similar to female, but wings hyaline and antennal flagellum composed of a single elongate clava.

DNA diagnosis: The 28S D2-D3 expansion region was sequenced for 745 bp for the forward sequence but only partially (486 bp) for the reverse sequence. Hence a viable contig could only be obtained using very high levels of tolerance of low quality. Nevertheless the forward sequence BLASTed to *A. arizonensis* (three top hits) had 99.8% pairwise identity. The reverse sequence BLASTed to the same three *A. arizonensis* sequences had only 85% pairwise identity. Unsurprisingly, the contig of these two sequences

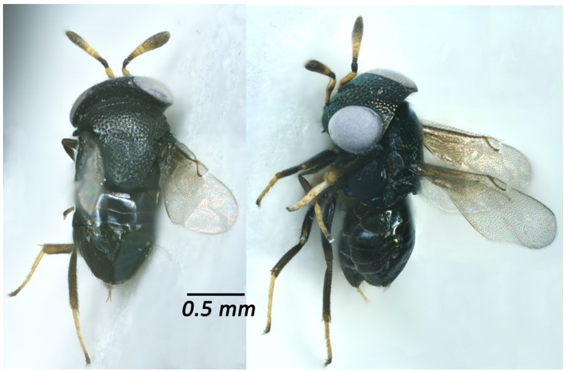

Fig. 1 *Aenasius arizonensis* female, dorsal and lateral views

Fig. 2 *Aenasius arizonensis* male, dorsal and lateral views

Fig. 3 *Cheiloneurus nankingensis* female, dorsal and lateral views

(753 bp) BLASTed to *A. arizonensis* (three top hits) had 89.4% pairwise identity, and 84.3% identical sites. Because of the disparity in GenBank between this sequence and the closest hit (MT783481.1), despite the positive identification, it was not possible to deposit this sequence in GenBank.

Hosts: Phenacoccus solenopsis; *Pseudococcus longispinus*.

Distribution: China, Egypt (Attia et al. 2017), India, Iraq, Iran, Pakistan, Turkey. First recorded in Iraq from Baghdad (Abdul-Rassoul 2018).

Notes: A. arizonensis is known as a solitary nymphal endoparasitoid (Noyes and Ren 1995; Poorani et al. 2009; Solangi and Mahmoud 2011). In India it has been known to cause more than 80% percent parasitoidism of *P. solenopsis* (Ram et al. 2009).

Material examined: Five♀, six ♂. Iraq, Karbala Governorate, 32° 36′ 40.2″ N, 44° 01′ 25.2″ E, 06/2023, col. AH Khudhair. Voucher specimens deposited at NHMUK (1♀ DNA1843); and Kerbala University Insect Collection.

Cheiloneurus nankingensis Li and Xu

Cheiloneurus nankingensis Li and Xu (Li et al. 2020, p. 25–27). http://zoobank.org/0A0CAA90-9BAD-4A04-8E4A-64851CE36387.

Morphological diagnosis: Female (Fig. 3): General body colour brassy-yellow, the mesoscutum and T1 of metasoma metallic blue-green, metanotum and propodeum metallic brown. Antennal scape and pedicel not flattened and expanded, F1 and F2 longer than broad, remaining antennomeres transverse, and giving the flagellum a very striking flattened appearance (Fig. 3), apex of clava with an oblique truncation, fully winged, fore wings passing apex of metasoma, funicle 6-merous, fore wing centrally strongly and continuously infuscate, patch of setae basal to linea calva and including filum spinosum comprising 8–14 setae in two rows, mesoscutellum with a tuft of hairs.

DNA diagnosis: The species was identified from a single Iraq male whose forward (634 bp) and reverse (788 bp) fragments produced a contig with 94.59% identity with *Cheiloneurus fulvescens* when BLASTed. A female and male specimen from Gebiz, Serik, Turkey (ex *P. solenopsis*), produced CO1 sequences identical to each other, and to the Iraq male. These sequences have been deposited in GenBank under the following Accession Nos PQ358949; PQ358950; PQ352466. Morphologically, there was extremely strong agreement with the very detailed descriptions of *C. nankingensis*, reared from the same host (Li et al. 2020). These are the first records of this species from Iraq and Turkey.

Cheiloneurus nankingensis closely resembles *C. compressicornis* (Ashmead), especially in the female. Females of the former differ from the latter in having the general body colour brassy-yellow (Fig. 3), while this is orange in *C. compressicornis* (Fig. 4). The basal hind tarsomere is a unicolorous dusky yellow in the former, and dark brown proximally in the latter. The shape of the female head also differs, with *C. nankingensis* narrower (length:width 50:40; 47:42 in *C. compressicornis*). Furthermore, the patch of fore

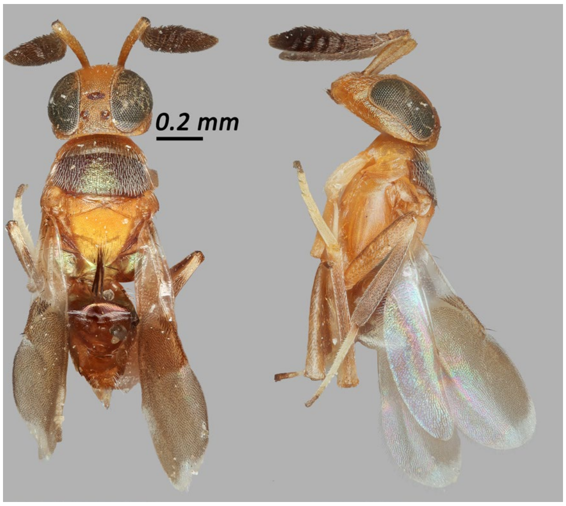

Fig. 4 *Cheiloneurus compressicornis* female, dorsal and lateral views (syntype)

Fig. 5 *Cheiloneurus nankingensis* male, dorsal and lateral views

wing setae proximal to the linea calva, including the filum spinosum, comprises 8–14 setae in two rows in *C. nankingensis* and > 20 in 5–6 rows in *C. compressicornis*. The males of the two species are strikingly different, with dark metallic blue-green as the ground colour in *C. nankingensis* (Fig. 5), and orange-brown as the ground colour in *C. compressicornis* (Fig. 6). In addition, the fore wing is hyaline in *C. nankingensis* and infuscate in *C. compressicornis*. Both species appear to belong to a group of closely related species in which the females appear rather similar, but in which the males differ considerably from each other and from their conspecific females in characters such as the presence of the hair tuft on the mesoscutellum, infuscation below the marginal vein, and especially body colour. *C. compressicornis* is a parasitoid of Chrysopidae coccoons (Neuroptera), and Chrysopidae are often found in association with mealybug colonies as predators. Given the distinct morphology of this species group, comprising *C. compressicornis*, *C. dubius* Howard, *C. nankingensis* and *C. repola* Noyes (Noyes 2017), and their association with hosts of known SW USA origin (*A. arizonensis*, *P. solenopsis*) it seems likely that *C. nankingensis* is also of South-West USA origin, and not Asian in origin as implied by Qin et al. (2023). In all probability it has been overlooked, and/or misidentified, as *C. compressicornis* in the past.

Host: *Aenasius arizonensis*.

Distribution: China, Iraq, Turkey.

Notes: *C. nankingensis* is known so far as a parasitoid of *A. arizonensis* only.

Material examined: One ♂ Iraq: Karbala Governorate, 32° 36′ 40.2″ N, 44° 01′ 25.2″ E, 15/03/2023, AH Khudhair col. ex *Phenacocus solenopsis*. Voucher specimen deposited in NHMUK DNA 1842; one ♀, one ♂ Turkey: Serik, Gebiz, 22/06/2023 N. Topakcı col. ex *Phenacoccus solenopsis*. Voucher specimens deposited in NHMUK (one ♀ DNA1896; one ♂ DNA1897).

Fig. 6 *Cheiloneurus compressicornis* male, dorsal and lateral views (syntype)

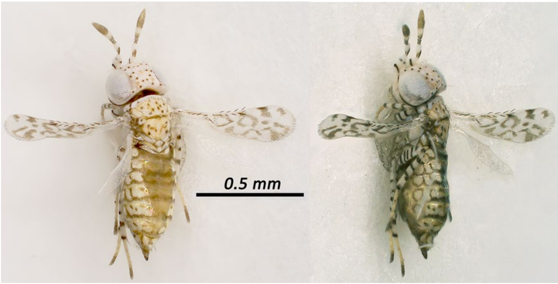

Fig. 7 *Marietta pictus* female, dorsal and lateral views

Fig. 8 *Marietta pictus* male (brachypterous), dorsal and lateral views

Marietta pictus (André)

Agonioneurus pictus (André 1878, p. 85).

For synonymy see Universal Chacidoidea Database at: https://ucd.chalcid.org/#/taxon?taxonID=462121.

Morphological diagnosis: Female (Fig. 7): Antenna 6-merous, fore wing with a pattern of infuscation bearing dark setae, and hyaline areas with colourless setae. Antennal scape flattened, about three times as long as maximum width; mid tibia with two complete fuscous bands (Hayat 2009). Males have the apical antennomere longer than in the female, and are often brachypterous (short-winged, Fig. 8).

DNA diagnosis: DNA was extracted from a single pupa ex *P. solenopsis* from Kerbala, Iraq. Forward and reverse 28S D2 sequences were obtained, which after alignment and trimming of the contig (740 bp) BLASTed to *Marietta pictus* in GenBank with 98.87 percentage identity (100% query cover). Despite the absence of a recognisable specimen, we consider this solely DNA-based identification as highly plausible, and here consider *Marietta pictus* to be a newly recorded hyperparasitoid of *P. solenopsis*, recorded from Iraq for the first time. The sequence has been deposited in GenBank under Accession No. PQ355529.

Hosts: Universal Chalcidoidea Database records 29 secondary hosts (aphids, psyllids and scale insects) and 21 primary hosts (other parasitoids) of *M. pictus* https://ucd.chalcid.org/#/taxon?taxonID=462121.

Distribution: Universal Chalcidoidea Database records *M. pictus* from much of the Palaearctic, as well as Canada, Mexico and Colombia, but not the USA.

Material examined: single pupa, sex not determined, post DNA-extraction: Iraq, Karbala Governorate, 32° 36′ 40.2″ N, 44° 01′ 25.2″ E, 06/2023, col. AH Khudhair. Voucher specimen deposited at NHMUK (DNA1844).

Discussion

Three parasitoids, two of which are new for the fauna of Iraq, are recorded herein, associated with the invasive North American cotton mealybug, *Phenacoccus solenopsis*. It appears probable that *Aenasius arizonensis* and its primary parasitoid *Cheiloneurus nankingensis*, have been accidentally introduced with the host. Despite having been recorded from China, evidence from its host association, morphology and DNA, point to *C. nankingensis* having originated in North America. *Marietta pictus*, on the other hand, is long known as a species of Palaearctic origin, probably having been introduced into the New World, though as yet unrecorded from USA. In the present context, it appears therefore to have been "recruited" from local populations originally on other hosts by the invasive mealybug.

The somewhat fortuitous discovery of *M. pictus* from a single unrecognisable pupa emphasises the pressing need for more complete molecular databases, such as NCBI GenBank and BOLD, as stressed by Miller et al (2021). Without a high quality 28S D2-D3 sequence in GenBank of *M. pictus*, identification of that species in this project would not have been possible. Existing sequences of *A. arizonensis* were very useful in corroborating morphological diagnoses. Finally, despite the nearest "hit" in GenBank for *Cheiloneurus nankingensis* being 95% similarity, the correct genus was confirmed, which in the family Encyrtidae is by no means straightforward, particularly when dealing with an isolated male.

Hyperparasitoids have generally been considered as necessarily detrimental to biocontrol or natural control scenarios based on the obvious fact that they kill the beneficial primary parasitoids. There is, however, evidence to the contrary, which indicates that the presence of hyperparasitoids in a given trophic system dampens extreme population swings between the target host and primary parasitoid, thus engendering greater stability in the system. Hyperparasitoidism promotes parasitoid coexistence by attacking the most prevalent parasitoid species, and this has been demonstrated for *Marietta pictus*, one of our study species (Wu et al. 2018). When hyperparasitoids attack parasitoids associated with plant feeders in different food web components, they impact competition (Morris et al. 2001). Absence of hyperparasitoids in the system can cause secondary extinctions of the plant-feeders and their primary parasitoids (Poelman et al. 2022). The results of this project are not only of importance for the continuing development of strategies to combat invasive pests such as cotton mealybug, but underline the critical importance of meticulous and mutually verifiable diagnostic approaches using molecules and morphology.

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Declarations

Competing interests None of the authors have any potential conflicts of interest.

Informed consent We declare no issues of informed consent were involved in this research.

Research involved in human and animal participants This research involved no human or other animal participants other than the insects involved, which were collected humanely.

References

Abdul-Rassoul MS (2018) First record of *Aenasius arizonensis* (Girault, 1915) (Hymenoptera, Encyrtidae), a parasitoid of *Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera, Pseudococcidae) in Iraq. Bull Iraq Nat Hist Mus 15(1):93–100

Abdul-Rassoul MS, Al-Mallo IM, Hermiz FB (2015) First record of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera, Pseudococcidae) from Iraq. JBES 7(2):216–222

Afzal M, Rehman SU, Siddiqui MT (2009) Appearance and management of a new devastating pest of cotton *Phenacoccus solenopsis* Tinsley, in Pakistan. Beltwide cotton conference, San Antonio, Texas, pp 5–8

André E (1878) Mémoire pour servir à l'histoire de la *Trioza centrathi* Vallot. Ann Soc Entomol Fr 8:77–86

Attia AR, Awadalla KT, Noyes JS (2017) First record of *Aenasius arizonensis* (Hymenoptera; Encyrtidae) as a solitary, endoparasitoid of cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera; Pseudococcidae) infesting different host plants at Giza region in Egypt. Egypt Acad J Biol Sci A Entomol 10(7):127–134

Beasley J (2023) DNA barcoding protocol for Darwin tree of life at the natural history museum of London. Protocols. https://doi.org/10.17504/protocols.io.8epv5x7qjg1b/v1

Chen HY, Li HL, Pang H, Zhu CD, Zhang YZ (2021) Investigating the parasitoid community associated with the invasive mealybug *Phenacoccus solenopsis* in Southern China. Insects 12(4):290

Cruaud A, Nidele S, Arnal P, Weber A, Fusu L, Gumovsky A, Huber J, Polaszek A, Rasplus JY (2019) Optimized DNA extraction and library preparation for minute arthropods: application to target enrichment in chalcid wasps used for biocontrol. Mol Ecol Resour 19:702–710

Culik MP, Gullan PJ (2005) A new pest of tomato and other records of mealybugs (Hemiptera: Pseudococcidae) from Espírito Santo, Brazil. Zootaxa 964:1–8

Fallahzadeh M, Japoshvili G, Abdimaleki R, Saghaei N (2014) New records of Tetracneminae (Hymenoptera, Chalcidoidea, Encyrtidae) from Iran. Turk J Zool 38:515–518

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3(5):294–299

Fusu L, Polaszek A (2017) Description, DNA barcoding and phylogenetic placement of a remarkable new species of *Eopelma* (Hymenoptera: Eupelmidae) from Borneo. Zootaxa 4263:557–566

García Morales M, Denno BD, Miller DR, Miller GL, Ben-Dov Y, Hardy NB (2016) ScaleNet: a literature-based model of scale insect biology and systematics. Database. https://scalenet.info/. Accessed 5 Aug 2024

Girault AA (1915) New chalcidoid Hymenoptera. Ann Entomol Soc Am 8:279–284

Hayat M (2009) Description of a new species of *Aenasius* Walker (Hymenoptera: Encyrtidae), parasitoid of mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae). Biosystematica 3:21–25

Hodgson CJ, Abbas G, Arif MJ, Saeed S, Karar H (2008) *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), a new invasive mealybug damaging cotton in Pakistan and India with a discussion on seasonal morphological variation. Zootaxa 1913:1–35

Jhala RC, Bharpoda TM, Patel MG (2008) *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), the mealybug species recorded first time on cotton and its alternate host plants in Gujarat. India UPJOZ 28(3):403–406

Li Z, Yao T, Xu Z, Meng L, Li BP (2020) A new species of *Cheiloneurus* Westwood (Hymenoptera, Encyrtidae) as a hyperparasitoid of the invasive cotton mealybug, *Phenacoccus solenopsis* Tinsley, in China. ZooKeys 974:23–29

Miller KE, Polaszek A, Evans DM (2021) A dearth of data: fitting parasitoids into ecological networks. Trends Parasitol 37(10):863–874

Morris RJ, Muller CB, Godfray HCJ (2001) Field experiments testing for apparent competition between primary parasitoids mediated by secondary parasitoids. J Anim Ecol 70:301–309

Muniappan R (2010) Three alien invasive mealybugs are on the march in Asia. IAPPS, No. X, October 2010

Noyes JS (1982) Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). J Nat Hist 16:315–334

Noyes JS (2017) Universal Chalcidea database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids. Accessed 6 May 2017

Noyes JS, Ren H (1995) Encyrtidae of Costa Rica (Hymenoptera: Chalcidoidea): the genus *Aenasius* Walker, parasitoids of mealybugs (Homoptera: Pseudococcidae). Bull Br Mus Nat Hist Entomol 64(2):117–163

Noyes JS, Woolley JB (1994) North American encyrtid fauna (Hymenoptera: Encyrtidae): taxonomic changes and new taxa. J Nat Hist 28(6):1327–1401

Park JK, O'Foighil D (2000) Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. Mol Phylogenet Evol 14:75–88

Poelman EH, Cusumano A, de Boer JG (2022) The ecology of hyperparasitoids. Annu Rev Entomol 67:143–161

Poorani J, Rajeshwari SK, Gupta A (2009) Notes on diagnosis and biology of *Aenasius bambawalei* Hayat (Encyrtidae: Hymenoptera) a parasitoid of the invasive mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Sternorrhyncha: Pseudococcidae). JBC 23(4):463–466

Qin W, Lyu Y, Yao T, Meng L, Li B (2023) Host stage affects oviposition-related behaviours, development progression and reproductive output in a native hyperparasitoid of the solenopsis mealybug invading Asian regions. Biocontrol Sci Technol 33:789–804

Ram P, Saini RK, Vijaya (2009) Preliminary studies on field parasitisation and biology of solenopsis mealybug parasitoid *Aenasius bambawalei* Hayat, (Encyrtidae: Hymenoptera). JCRD 23:313–315

Sanaa AM, El-Mageed A, SoadI AR, Harris M (2020) Ecological studies on the cotton mealybug *Phenacoccus solenopsis* (Hemiptera; Pseudococcidae) on maize in Upper Egypt. EJPP 3(4):1098–1110

Sirisena UGAI, Watson GW, Hemachandra KS, Wijayagunasekara HNP (2013) A modified technique for the preparation of specimens of Sternorrhyncha for taxonomic studies. J Trop Agric 24(2):139–149

Solangi GS, Mahmood R (2011) Biology, host specificity and population trends of *Aenasius bambawalei* Hayat and its role controlling mealybug *Phenacoccus solenopsis* Tinsley at Tandojam Sind. 5th Meeting Asian Cotton Research and Development Network held on February 23–25 Lahore, pp 1–7

Srivathsan A, Lee L, Katoh K, Hartop E, Kutty SN, Wong J, Yeo D, Meier R (2021) ONTbarcoder and MinION barcodes aid biodiversity discovery and identification by everyone, for everyone. BMC Biol 19(1):217

Wang YP, Wu SA, Zhang RZ (2009) Pest risk analysis of a new invasive pest, *Phenacoccus solenopsis* to China. Chin J Entomol 46:101–106

Whiting MF, Carpenter JC, Wheeler QD, Wheeler WC (1997) The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst Biol 46:1–68

Wu P, Ma B, Yan S, Xu J, Zhang R (2018) The hyperparasitoid *Marietta picta* (Hymenoptera: Aphelinidae) mediates competitive interactions between two parasitoids of *Paratrioza sinica* (Hemiptera: Psyllidae): *Tamarixia lyciumi* (Hymenoptera: Eulophidae) and *Psyllaephagus arenarius* (Hymenoptera: Encyrtidae). Biol Control 126:169–176

Zhao J, Watson GW, Sun Y, Tan YG, Xiao LB, Bai LX (2014) Phenotypic variation and identification of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in China. Zootaxa 3802(1):109–121

Zhu Y, Huang F, Lu Y (2011) Bionomics of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on cotton. Acta Entomol 54(2):246–252

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