



Article Investigating the Parasitoid Community Associated with the Invasive Mealybug *Phenacoccus solenopsis* in Southern China

Hua-Yan Chen^{1,2}, Hong-Liang Li¹, Hong Pang², Chao-Dong Zhu¹ and Yan-Zhou Zhang^{1,*}

- Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China; chenhuayan@mail.sysu.edu.cn (H.-Y.C.); lihongliang_2019@163.com (H.-L.L.); zhucd@ioz.ac.cn (C.-D.Z.)
- ² State Key Laboratory of Biocontrol, Ecology and Evolution, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China; Lsshpang@mail.sysu.edu.cn
- Correspondence: zhangyz@ioz.ac.cn

Simple Summary: The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is an emerging invasive insect pest in China. Hymenopteran parasitoids have great potential to be used as biological agents to suppress the populations of *P. solenopsis* in the field. We used an integrated approach of species delimitation, combining morphology with molecular data, to investigate the parasitoid community associated with *P. solenopsis* from south China. On the basis of both morphological and molecular evidence, we identified 18 hymenopteran parasitoid species belonging to 11 genera of four families. Among these species, eight species are primary parasitoids with *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae) being the dominant taxon, while the other 10 species are probably hyperparasitoids. These findings can assist in the utility of these parasitoids in the biological control of *P. solenopsis*.

Abstract: The cotton mealybug Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae), is an emerging invasive insect pest in China. Hymenopteran parasitoids are the key organisms for suppressing populations of *P. solenopsis* in the field, and therefore could be used as biological agents. Accurate identification of the associated parasitoids is the critical step to assess their potential role in biological control. In this study, we facilitated the identification of the parasitoid composition of P. solenopsis using an integrated approach of species delimitation, combining morphology with molecular data. Eighteen Hymenoptera parasitoid species belonging to 11 genera of four families are recognized based on morphological examination and molecular species delimitation of the mitochondrial cytochrome c oxidase 1 (COI) gene and the 28S rDNA using the automatic barcode gap discovery (ABGD) and the Bayesian Poisson tree processes model (bPTP). Among these species, eight species are primary parasitoids with Aenasius arizonensis (Girault) (Hymenoptera: Encyrtidae) being the dominant taxon, while the other 10 species are probably hyperparasitoids, with a prevalence of Cheiloneurus nankingensis Li & Xu (Hymenoptera: Encyrtidae). These results indicate that parasitoid wasps associated with P. solenopsis from China are diverse and the integrated taxonomic approach applied in this study could enhance the accurate identification of these parasitoids that should be assessed in future biological control programs.

Keywords: biological control; DNA barcoding; integrated taxonomy; species delimitation

1. Introduction

The mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), which originating from the USA, has become an emerging invasive and polyphagous pest in Asia [1–6]. It has been reported to feed on at least 213 host plant species belonging to 56 families [7], including the economically important cotton [5,6,8]. This mealybug species has caused serious yield losses of cotton in some Asian countries, particularly India and Pakistan [9,10]. Since its first report in mainland China in 2008, this mealybug has spread to



Citation: Chen, H.-Y.; Li, H.-L.; Pang, H.; Zhu, C.-D.; Zhang, Y.-Z. Investigating the Parasitoid Community Associated with the Invasive Mealybug *Phenacoccus solenopsis* in Southern China. *Insects* **2021**, *12*, 290. https://doi.org/ 10.3390/insects12040290

Academic Editor: Alberto Alma

Received: 24 February 2021 Accepted: 23 March 2021 Published: 26 March 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). 16 provinces of the country by the end of 2018 [11]. It was estimated that all cotton-growing regions of China could be infested by this mealybug [5].

Currently, chemical control is still the main strategy against *P. solenopsis* worldwide. However, due to the presence of a waxy coating over the body, ovoviviparous mode of reproduction (parthenogenesis with ovoviviparity is dominant over the oviparous mode), and overlapping generations and their cryptic habits, effective management of this pest is often limited to conventional insecticides [12]. In addition, repeated and overuse of insecticides have resulted in resistance and tolerance of *P. solenopsis* to some insecticides, and a negative impact on natural enemies, which could cause the resurgence of this pest [13–16]. Therefore, strategies for sustainable management of *P. solenopsis*, including biological control, have attracted increasing attention in recent years. Biological control involving natural enemies, especially parasitoids, has been thought to be the key factor in suppressing the populations of *P. solenopsis* in nature [12]. At least 38 species of parasitoids or hyperparasitoids (Table 1) belonging to six hymenopteran families have been reported to be associated with *P. solenopsis* throughout its distribution range [17–29]. Further assessment and application of these parasitoid wasps in biological control programs against P. solenopsis require an accurate identification of the species [28]. However, given the diverse guild of wasps associated with *P. solenopsis*, species identification based on morphology is not easy to handle, particularly for practitioners who are not the specialists of parasitoids, because these parasitoids are usually small and morphologically similar and require extensive examination of detailed morphological characters. DNA barcoding (the partial sequencing of the gene cytochrome c oxidase 1, COI) has become a sufficient species identification tool for insects [30,31]. Studies have shown that DNA barcoding is a useful tool for the identification of various groups of parasitoids [32–35]. In this study, we use an integrated approach (morphology combined with DNA barcoding) to identify the parasitoids associated with *P. solenopsis* from China. The results from this study may help to improve the determination of parasitoid species of *P. solenopsis*, which is a key step toward using parasitoids as biological control agents against this serious pest.

2. Materials and Methods

2.1. Parasitoid Collecting and Sampling

During 2010–2017, we surveyed the parasitoids of *P. solenopsis* in 9 provinces of southern China (Hainan, Guangdong, Guangxi, Jiangxi, Yunnan, Zhejiang, Fujian, Hunan and Hubei provinces). Branches and leaves of various host plants infested by *P. solenopsis* were collected from the field and brought back to the Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences (IZCAS), and kept in the greenhouse and checked daily for parasitoid emergence. Any parasitoids emerged were collected, preserved in 100% ethanol, and stored at -20 °C until further morphological and molecular studies. All specimens were identified to genus and provisional morphospecies. At least two specimens of each morphospecies from each locality were selected for DNA sequencing. After DNA extraction, the specimens were re-examined to confirm their morphological identification. All specimens (including DNA vouchers, Table S1) in this study are deposited at the Institute of Zoology, Chinese Academy of Sciences (IZCAS).

DNA Extraction, Amplification, and Sequencing

Overall, 212 representative specimens of 18 morphospecies were used for DNA barcoding analysis (see Table S2). Genomic DNA was extracted from each specimen using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. Polymerase chain reaction (PCR) amplification of two DNA fragments, mitochondrial DNA (mtDNA) cytochrome c oxidase 1 (*COI*) and nuclear 28S rRNA D1–2 (*28S*), were carried out on an Eppendorf thermal cycler and performed in 50 μ L volumes containing 5 μ L DNA template, 24.5 μ L ddH₂O, 5 μ L 10× buffer, 5 μ L MgCl₂, 8 μ L dNTP, 1 μ L of each primer, and 0.5 μ L LaTaq (Takara, Shiga, Japan). Primers [36–41], fragment length, and references are shown in Table S3. The PCR programs followed Chesters et al. [42]. The PCR products were visualized by 1% agarose gel electrophoresis. Products were purified and sequenced in both directions using BigDye v3.1 on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). Chromatograms were assembled with Sequencing Analysis 6 (ThermoFisher Scientific, Gloucester, UK). All the amplified sequences were deposited in GenBank (accession numbers in Table S2).

2.2. Sequence Analysis and Molecular Species Delimitation

All sequences were blasted in the BOLD (Barcode of Life Database, http://www. barcodinglife.org/index.php/IDS_OpenIdEngine) and GenBank. The blast hits with over 97% similarity were recorded. Sequences were aligned using MAFFT v7.470 by the Q-INS-I strategy for 28S and G-INS-I strategy for *COI* [43]. The two fragments were concatenated in Geneious 11.0.3. In the final 1302 bp concatenated alignment, 28S and *COI* were 672 bp and 630 bp, respectively. Genetic Kimura-2 parameter (K2P) distances within and between species were calculated in MEGA 7 with pairwise deletion for gaps [44].

Distance and evolutionary model-based methods were both tested for molecular species delimitation. The automatic barcode gap discovery (ABGD) is a distance-based method that sorts the sequences into hypothetical species by partitioning and comparing the difference between sequences to identify a "barcode gap" [45]. The ABGD analyses were performed with the COI dataset on the web interface (http://wwwabi.snv.jussieu. fr/public/abgd/), using the default priors, Pmin = 0.001, Pmax = 0.1, Steps 10, and with barcode relative gap width = 1.00. The Poisson tree processes model (PTP) tests species boundaries on non-ultrametric phylogenetic trees by detecting significant differences in the number of substitutions between species and within species [46]; Bayesian Poisson tree processes model (bPTP) is an updated version of the original PTP with Bayesian posterior probability, providing more accurate results. For bPTP analyses, after removing the identical sequences, a maximum likelihood (ML) tree was generated in RAxML v8.2.10 under the GTRGAMMA evolutionary model and performed on the bPTP web server (https://species.h-its.org/ptp/), with default parameters. The COI and 28S sequences of Hybrizon buccatus (de Brebisson) (Hymenoptera: Ichneumonidae, GenBank: KU753286 (COI), KU753494 (28S)) were selected as the outgroup because of the close relationship between Ichneumonoidea and Chalcidoidea + Platygastroidea [47].

3. Results and Discussion

A total of 870 individual parasitoid wasps emerged from 62 samples of *P. solenopsis* infecting 11 plant species from nine provinces of southern China (Table S2). Of these specimens, the initial morphological identifications suggested a total of 18 morphospecies belonging to four hymenopteran families as follows: Aphelinidae (3): *Marietta picta* (André), *Myiocnema comperei* Ashmead, *Promuscidea unfasciativentris* Girault (Figure 1C); Encyrtidae (11): *Acerophagus* sp1, *Acerophagus* sp2, *Aenasius arizonensis* (Girault) (Figure 1A), *Anagyrus jenniferae* Noyes & Hayat, *Anagyrus kamali* Moursi, *Anagyrus tristis* Noyes & Hayat, *Cheiloneurus nankingensis* Li & Xu (Figure 1B), *Gyranusoidea indica* Shafee, Alam & Agarwal, *Prochiloneurus javanicus* (Ferriere), *Prochiloneurus stenopterus* Wang, Huang & Xu, *Prochiloneurus testaceus* (Agarwal); Platygasteridae (1): *Allotropa phenacocca* Chen, Liu & Xu; Signiphoridae (3): *Acerophagus* sp1, *Acerophagus* sp2, *Acerophagus* sp3. (Table S1, Figure 2). Apparently, Encyrtidae species are predominant, comprising 61% of the parasitoid community found in southern China. The present study generated DNA sequences for 212 specimens, with matrices that ranged from 468 to 582 bp for 28S, and from 561 to 618 bp for COI.



Figure 1. (**A**) Aenasius arizonensis parasitizing Phenacoccus solenopsis; (**B**) *Cheiloneurus nankingensis* parasitizing Phenacoccus solenopsis via probably Aenasius arizonensis; (**C**) Promuscidea unfasciativentris parasitizing a mummy of Phenacoccus solenopsis.



Figure 2. Maximum likelihood tree and results of species delimitation, only values > 90 for bootstrap are labeled.

3.1. BLAST in the NCBI and the Barcode of Life Database (BOLD)

The results of the sequence comparison against the known sequences in the BOLD system (only COI) and GenBank database (both 28S and COI) are listed in Table S4. The COI sequences of four species received "top hits" with different extents of similarity (over 98%) through the BOLD identification system. When blasted in GenBank database, four species returned close matches for COI, seven species returned close matches for 28S. Other species had no matching sequences in the BOLD or the NCBI.

3.2. Genetic Distances

The K2P distances (Tables S5–S8) indicated a larger intergroup than intragroup distance for both COI and 28S, and the mean genetic distance accrete with the improvement of classification. The mean intraspecific pairwise distance for COI was 0.33% (range 0–2.33%) and 0.02% (range 0–0.17%) for 28S; the mean interspecific pairwise distance for COI was 20.30% (range 9.38–35.53%) and 20.42% for 28S (range 3.25–39.04%). The mean intrageneric pairwise distance was 2.75% (range 0–8.73%) for COI and 1.50% (range 0–7.01%) for 28S; the mean intergeneric pairwise distance for COI was 21.62% (range 11.89–35.53%) and 22.03% (range 12.09–39.04%) for 28S. The mean intrafamily pairwise distance for COI was 9.38% (range 1.07–14.90%) and 7.60% (range 0.02–13.45%) for 28S; the mean interfamily pairwise distance for COI was 25.52% (range 18.11–33.67%) and 25.98% (range 16.13–36.75%) for 28S.

3.3. The Automatic Barcode Gap Discovery (ABGD) Analysis and Bayesian Poisson Tree Processes Model (bPTP)

The ABGD analyses returned a total of 18 groups both for 28S and COI at a priori genetic distance thresholds of 0.04–0.09, which are congruent with our identification based on morphology (Figure 2). After removing the identical sequences, 22 ingroup taxa of the concatenated data set (28S + COI) were included to construct the ML tree. The bPTP analysis based on this ML tree delimited 18 putative species, which are also congruent with the morphological identification results (Figure 2).

The 18 species of parasitoids we identified and described below.

3.3.1. Aphelinidae

Genus Marietta

Morphological characters and molecular data both strongly support the identity of *Marietta picta* (André) (emerged from *P. solenopsis* feeding on *Justicia gendarussa* Burm. f. (Acanthaceae), *Hibiscus rosa-sinensis* L. (Malvaceae) and *Solanum nigrum* L. (Solanaceae) in Guangxi and Fujian) in this study. This species has been recorded as parasitoids of *A. arizonensis* and *Prochiloneurus aegyptiacus* (Mercet, 1929) and hyperparasitoid of *P. solenopsis* [28].

Genus Myiocnema

Myiocnema comperei Ashmead (emerged from *P. solenopsis* feeding on *Ageratum conyzoides* L. (Asteraceae), *J. gendarussa*, *H. rosa-sinensis* and *S. nigrum* in Guangxi, Fujian and Hainan) was recognized based on morphological examination and molecular analyses. The COI sequence of this species showed a more than 98% identity to *Myiocnema comperei* Ashmead in the BOLD and the NCBI. This species has been recorded as hyperparasitoids of the primary parasitoids (unidentified) of Coccidae and Pseudococcidae from China [48].

Genus Promuscidea

Morphological characters and molecular data, in this study, both strongly support the identity of *Promuscidea unfasciativentris* Girault (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* and *S. nigrum* in Hainan). This species has been recorded as a hyperparasitoid of *P. solenopsis* [49].

3.3.2. Encyrtidae *Genus Acerophagus*

Two species of *Acerophagus* are recognized, which we temporarily named as *Acerophagus* sp1 (emerged from *P. solenopsis* feeding on *J. gendarussa* in Guangdong) and *Acerophagus* sp2 (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* and *Chaenomeles sinensis* (Thouin) Koehne (Rosaceae) in Hainan and Yunnan, respectively); only the 28S sequence of *Acerophagus* sp1 showed 99.8% identity to an undetermined *Acerophagus* species in the NCBI. *Acerophagus coccois* Smith was previously reared as the primary parasitoid of *P. solenopsis* in southern China [20,27]. However, none of the two species recognized in the present study is conspecific with *Ac. coccois*. Further taxonomic work is required for both species.

Genus Aenasius

In this study, only one species of *Aenasius, A. arizonensis*, is easy to recognize by its morphology, and the COI sequences of representative specimens matched with those of *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) both in the BOLD and in the NCBI (Table S4). *Aenasius arizonensis* seems to be the dominant primary parasitoid of *P. solenopsis* in China [27,50,51]. In our study, specimens of *A. arizonensis* comprise 56% of the total number of all parasitoid species and this species emerged from *P. solenopsis* feeding on 11 plant species in all the nine provinces surveyed. Abundant studies have shown that *A. arizonensis* is a potent parasitoid against *P. solenopsis* [10]. Developing efficient mass rearing and releasing methods could be the focus to use this species as a biological agent in future research.

Genus Anagyrus

The following three species of *Anagyrus* are recognized: *An. jenniferae* (emerged from *H. rosa-sinensis* in Guangdong, Guangxi, and Hainan), *An. kamali* (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* in Hainan) and *An. tristis* (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* and *Ficus microcarpa* L. (Moraceae) in Hainan). The ABGD and bPTP analyses also both supported a three-species scenario among the studied specimens. For the blast in the BOLD and in the NCBI, only the 28S of *An. kamali* recovered a high match in the NCBI. A number of *Anagyrus* species have been reported to attack *P. solenopsis* [19,27,28,52]. Considering the extreme morphological similarity among these species, a DNA barcode reference library of these species should enhance their accurate identifications in future surveys.

Genus Cheiloneurus

The morphological characters and molecular data both indicate that one species of Cheiloneurus (emerged from *P. solenopsis* feeding on *Achyranthes bidentata* Blume (Amaranthaceae), *Ag. conyzoides*, *H. rosa-sinensis* and *Portulaca grandiflora* Hook (Portulacaceae) in Fujian and Jiangxi) is present in this study. We identified the species as *Cheiloneurus nankingensis* Li & Xu based on morphology. This species was first found from Guangxi (then reported as an undescribed species, confirmed by HYC) and was erroneously reposted as a primary and dominant parasitoid of *P. solenopsis* in China. Recently, Li et al. [29] described *C. nankingensis* as a new species and confirmed that it is a hyperparasitoid with *A. arizonensis* as the host. Preliminary observation shows that this species is a gregarious parasitoid, a few and up to 18 individuals have been found emerged from a single mummy of *P. solenopsis* [27,29]. The astonishing reproduction capacity and high parasitism rate of *A. arizonensis* in biological programs. In addition to this study, *C. nankingensis* has been reported from southern to eastern China. The prevalence of hyperparasitism by *C. nankingensis* across the range of *P. solenopsis* requires further investigation [29].

Genus Gyranusoidea

The morphological and molecular data both indicate that only one species of *Gyranu*soidea (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* in Hainan) is present. We identified the species as *Gyranusoidea indica* Shafee, Alam & Agarwal based on morphology. There was no blast match recovered in the BOLD or in the NCBI. *Gyranusoidea indica* has been recorded as a parasitoid of several other mealybug species and used in biological control programs against the pink hibiscus mealybug [53]. In this study, for the first time, we record this species as a parasitoid of *P. solenopsis*.

Genus Prochiloneurus

The following three species of *Prochiloneurus* are recognized: *Pr. javanicus* (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* in Guangxi, Yunnan and Hainan), *Pr. testaceus* (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* in Yunnan) and *Pr. stenopterus* (emerged from *P. solenopsis* feeding on *Ag. conyzoides* in Fujian). In addition, the ABGD and bPTP analyses both support a three-species scenario among the studied specimens, but there was no blast match recovered in the BOLD or in the NCBI. Species of *Prochiloneurus* are mainly hyperparasitoids of Coccidae and Pseudococcidae (Hemiptera) via other Encyrtidae species [54–58]. *Prochiloneurus stenopterus* has been previously recorded as a parasitoid of *A. arizonensis* in China [58]. *Prochiloneurus javanicus* and *Pr. testaceus* have been reported as hyperparasitoids of *An. kamali* [59] and *Anagyrus dactylopii* (Howard) [60], respectively.

3.3.3. Platygasteridae

Genus Allotropa

The morphological characters and molecular data both indicate that only one species of *Allotropa* (emerged from *P. solenopsis* feeding on *H. rosa-sinensis* in Guangxi and Hainan) is present and we identify the species as *Allotropa phenacocca* Chen, Liu & Xu based on morphology. There was no blast match recovered in the BOLD or in the NCBI. This species has been reported as an endoparasitoid of *P. solenopsis* from China [21,27].

3.3.4. Signiphoridae

Genus Chartocerus

Three species of *Chartocerus* are recognized, which we temporarily named *Chartocerus* sp1 (emerged from *P. solenopsis* feeding on *Ag. conyzoides* in Fujian), and *Chartocerus* sp3 (emerged from *P. solenopsis* feeding on *Ag. conyzoides* in Fujian). The 28S sequences of *Chartocerus* sp1 and *Chartocerus* sp2 showed more than 98% identity to undetermined *Chartocerus* species in the NCBI. The 28S sequences of *Chartocerus* sp1 and the COI sequences of all three species showed 90–92% identity to undetermined *Chartocerus* species in the NCBI. The 28S sequences are mainly known as hyperparasitoids of scale insects, mealybugs, and whiteflies (Hemiptera, Sternorrhyncha) through encyrtid or aphelinid primary parasitoids [61]. Our current study cannot verify their primary hosts in China, further studies on their biology should be carried out in the future.

Family	Species	Parasitoid or Hyperparasitoid	Reference
	Marietta picta (André, 1878)	parasitoid	[27,28]
Aphelinidae	Myiocnema comperei Ashmead	parasitoid	[27]
	Promuscidea unfasciativentris Girault	hyperparasitoid	[19,27]
Encyrtidae	Aenasius arizonensis (Girault)	parasitoid	[18]
	Acerophagus coccois Smith	parasitoid	[20,27]
	Acerophagus sp1	parasitoid	This study
	Acerophagus sp2	parasitoid	This study
	Anagyrus kamali Moursi	parasitoid	[19,27]
	Anagyrus agraensis Sarawat	parasitoid	[28]
	Anagyrus aligarhensis Agarwal and Alam	parasitoid	[28]
	Anagyrus californicus (Compere)	parasitoid	[52]
	Anagyrus dactylopii (Howard)	parasitoid	[19]
	Anagyrus jenniferae Noyes & Hayat	parasitoid	This study
	Anagyrus kamali Moursi	parasitoid	This study
	Anagyrus mirzai Agarwal & Alam	parasitoid	[19]
	Anagyrus osmoi Guerrieri & Ghahri	parasitoid	[62]
	Anagyrus tristis Noves & Havat	parasitoid	This study
	Bothriothorax serratellus (Dalman)	hyperparasitoid	[28]
	Cheiloneurus sp.	parasitoid	[17]
	Cheiloneurus nankingensis Li & Xu	hyperparasitoid	[27.29]
	Encurtus aurantii (Geoffroy)	parasitoid	[19]
	Guranusoidea indica Shafee, Alam & Agarwal	parasitoid	This study
	Homalotulus albiclavatus (Agarwal)	parasitoid	[19]
	Lentomastix algirica Trianitzin	parasitoid	[52]
	Lentomastix dactulonii Howard	parasitoid	[28]
	Leptomastix mauri Ózdikmen	parasitoid	[28]
	Lentomastix mayri Ozdikinch	parasitoid	[26]
	Metanhucus sp	parasitoid	[20]
	Prochiloneurus geometiacus (Morcot)	hyperparasitoid	[22]
	Prochiloneurus iavanicus (Wereet)	hyperparasitoid	[20]
	Prochiloneurus jacancias (Ferriere)	hyperparasitoid	[05], 1115 Study
	Prochuoneurus nugusukiensis (Istili)	hyperparasitoid	[20]
	Prochiloneurus migricornis (Girault)	hyperparasitoid	[27]
	Prochuloneurus puicheuus Silvestri	hyperparasitoid	[19,36]
	Prochilonaurus stanontorus Wang, Huang & Yu	hyperparasitoid	[40]
	Prochuloneurus sienopierus wang, Huang & Xu	hyperparasitoid	[56]; This study
	Prochiloneurus testuceus (Agarwai)	hyperparasitoid	Inis study
	Prochuoneurus uyguni Hayat	nyperparasitoid	[20] This studes
	Prochuoneurus sp.	? hyperparasitoid	Ins study
	Fseudieptomustix squammuutu Giraun	parasitolu	[32]
Eulophidae	Aprostocetus bangaloricus Narendran	parasitoid	[19]
	Aprostocetus minutus (Howard)	parasitoid	[17]
Platygastridae	Allotropa phenacocca Chen, Liu & Xu	parasitoid	[21,27]
Pteromalidae	Pachyneuron leucopiscida Mani	parasitoid	[19]
Signiphoridae	Chartocerus kerrichi (Agarwal)	parasitoid	[19]
	Chartocerus kurdjumovi (Nikolskaya)	hyperparasitoid	[28]
	Chartocerus sp1	?hyperparasitoid	This study
	Chartocerus sp2	?hyperparasitoid	This study
	Chartocerus sp3	?hyperparasitoid	This study
	*	** *	

Table 1. Hymenoptera parasitoids associated with *P. solenopsis* (the symbol "?" indicates that the biology is uncertain).

4. Conclusions

The parasitoid wasps associated with *P. solenopsis* from China are diverse. Our analyses based on both morphology and molecular data revealed 18 species belonging to 11 genera of four Hymenoptera families. Eight species of these wasps are primary parasitoids of *P. solenopsis* and some dominant species such as *A. arizonensis* have the potential to be developed as potent biological agents, while the other 10 species are hyperparasitoids

such as *C. nankingensis*, which may diminish the parasitism of the primary parasitoids of *P. solenopsis*. The integrated taxonomic approach applied in the present study to investigate the parasitoid community associated with *P. solenopsis* should enhance the accurate identification of these parasitoids, which is of great importance for developing an effective biological control program for this invasive pest in China. Moreover, this study contributes to further understanding some of the ecological interactions between the invasive *P. solenopsis* and the native associated parasitoids.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/insects12040290/s1. Table S1. Details of the sampling, host plants and number of parasitoids collected; Table S2. Details of the sampling and accession numbers of parasitoids sequenced; Table S3. Primers used to amplify two loci in this study; Table S4. Results of molecular analyses by ABGD, bPTP, BOLD (Barcode of Life Database) blast and NCBI blast (target species with similarity value less than 97% are not shown); Table S5. Genetic distances between parasitoid species between 28S sequences under K2P model; Table S6. Genetic distance within parasitoid species under K2P model; Table S8 K2P pairwise distances (%) of the COI and 28S gene within different taxonomic levels of the investigated parasitoids.

Author Contributions: Conceptualization, H.-Y.C., H.-L.L., and Y.-Z.Z.; methodology, H.-Y.C., H.-L.L., and Y.-Z.Z.; investigation, H.-Y.C., H.-L.L., and Y.-Z.Z.; writing—original draft preparation, H.-Y.C., H.-L.L., and Y.-Z.Z.; writing—review and editing, H.-Y.C., H.-L.L., H.P., C.-D.Z., and Y.-Z.Z.; supervision, H.P., C.-D.Z., and Y.-Z.Z.; funding acquisition, C.-D.Z. and Y.-Z.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This study is supported by the National Natural Science Foundation of China (NSFC grant no. 31872296 and 31572296).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data of the research were deposited in the Institute of Zoology, Chinese Academy of Scineces (IZCAS).

Acknowledgments: We would like to acknowledge our colleagues who assisted us with the collection of the specimens in the present paper.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- 1. Miller, D.R.; Miller, G.L.; Watson, G.W. Invasive species of mealybugs (Hemiptera: Pseudococcidae) and their threat to US agriculture. *Proc. Entomol. Soc. Wash.* **2002**, *104*, 825–836.
- Ben-Dov, Y. Note: The solanum mealybug, *Phenacoccus solani* ferris (Hemiptera: Coccoidea: Pseudococcidae), extends its distribution range in the mediterranean basin. *Phytoparasitica* 2005, 33, 15–16. [CrossRef]
- Wang, Y.P.; Wu, S.A.; Zhang, R.Z. Pest risk analysis of a new invasive pest, Phenacoccus solenopsis, to China. *Chin. J. Entomol.* 2009, 46, 101–106.
- Joshi, M.D.; Butani, P.G.; Patel, V.N.; Jeyakumar, P. Cotton mealy bug, Phenacoccus solenopsis Tinsley—A review. Agric. Rev. 2010, 31, 113–119.
- 5. Wang, Y.; Watson, G.W.; Zhang, R. The potential distribution of an invasive mealybug Phenacoccus solenopsis and its threat to cotton in Asia. *Agric. For. Entomol.* **2010**, *12*, 403–416. [CrossRef]
- 6. Tong, H.-J.; Ao, Y.; Li, Z.-H.; Wang, Y.; Jiang, M.-X. Invasion biology of the cotton mealybug, Phenacoccus solenopsis Tinsley: Current knowledge and future directions. J. Integr. Agric. 2019, 18, 758–770. [CrossRef]
- Abdulrassoul, M.S.; Almalo, I.M.; Hermiz, F.B. First record and host plants of Solenopsis Mealybug, Phenacoccus solenopsis Tinsley, 1898 (Hemiptera: Pseudococcidae) from Iraq. J. Biodivers. Environ. Sci. 2015, 7, 216–222.
- 8. Hanchinal, S.G.; Patil, B.V.; Basavanagoud, K.; Nagangoud, A.; Biradar, D.P.; Janagoudar, B.S. Incidence of invasive mealybug (Phenacoccus solenopsis Tinsley) on cotton. *Karnataka J. Agric. Sci.* **2011**, *24*, 143–145.
- Hodgson, C.; Abbas, G.; Arif, M.J.; Saeed, S.; Karar, H. Phenacoccus solenopsis Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. *Zootaxa* 2008, 1913, 1–35. [CrossRef]

- Nagrare, V.; Kranthi, S.; Biradar, V.; Zade, N.; Sangode, V.; Kakde, G.; Shukla, R.; Shivare, D.; Khadi, B.; Kranthi, K. Widespread infestation of the exotic mealybug species, Phenacoccus solenopsis (Tinsley) (Hemiptera: Pseudococcidae), on cotton in India. *Bull. Èntomol. Res.* 2009, *99*, 537–541. [CrossRef]
- Wang, Y.-S.; Dai, T.-M.; Tian, H.; Wan, F.-H.; Zhang, G.-F. Range expansion of the invasive cotton mealybug, Phenacoccus solenopsis Tinsley: An increasing threat to agricultural and horticultural crops in China. J. Integr. Agric. 2020, 19, 881–885. [CrossRef]
- 12. Fand, B.B.; Suroshe, S.S. The invasive mealybug Phenacoccus solenopsis Tinsley, a threat to tropical and subtropical agricultural and horticultural production systems—A review. *Crop. Prot.* **2015**, *69*, 34–43. [CrossRef]
- Kaur, H.; Virk, J.S. Feeding potential of Cryptolaemus montrouzieri against the mealybug Phenacoccus solenopsis. *Phytoparasitica* 2011, 40, 131–136. [CrossRef]
- Ejaz, M.; Ullah, S.; Shad, S.A.; Abbas, N.; Binyameen, M. Characterization of inheritance and preliminary biochemical mechanisms of spirotetramat resistance in Phenacoccus solenopsis Tinsley: An economic pest from Pakistan. *Pestic. Biochem. Physiol.* 2019, 156, 29–35. [CrossRef] [PubMed]
- Nagrare, V.S.; Fand, B.B.; Naik, V.C.B.; Naikwadi, B.; Deshmukh, V.; Sinh, D. Resistance development in Cotton mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) to insecticides from Organophosphate, Thiadiazines and Thiourea derivatives. Int. J. Trop. Insect Sci. 2019, 40, 181–188. [CrossRef]
- Afzal, M.B.; Shad, S.; Ejaz, M.; Serrao, J. Laboratory selection, cross-resistance, and estimations of realized heritability of indoxacarb resistance in Phenacoccus solenopsis (Homoptera: Pseudococcidae). *Pest Manag. Sci.* 2020, 76, 161–168. [CrossRef] [PubMed]
- 17. Fuchs, T.W.; Stewart, J.W.; Minzenmayer, R.; Rose, M. First record of Phenacoccus solenopsis Tinsley in cultivated cotton in the United States. *Southwest. Entomol.* **1991**, *16*, 215–221.
- 18. Hayat, M. Description of a new species of Aenasius Walker (Hymenoptera: Encyrtidae), parasitoid of the mealybug, Phenacoccus solenopsis Tinsley (Homoptera: Pseudococcidae) in India. *Biosystematica* 2009, *3*, 21–26.
- Vennila, S.; Ramamurthy, V.V.; Deshmukh, A.; Pinjarkar, D.B.; Agarwal, M.; Pagar, P.C.; Prasad, Y.G.; Prabhakar, M.; Kranthi, K.R.; Bambawale, O.M. *A Treatise on Mealybugs of Central Indian Cotton Production System*; Technical Bulletin no. 24; National Centre for Integrated Pest Management: New Delhi, India, 2010; 39p.
- 20. Chen, H.Y.; He, L.F.; Zheng, C.H.; Li, P.; Yi, Q.H.; Xu, Z.F. Survey on the natural enemies of mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) from Guangdong and Hainan, China. J. Environ. Entomol. 2011, 33, 269–272.
- 21. Chen, H.; Liu, J.; Xu, Z. Description of a new platygastrid parasitoidAllotropa phenacocca (Hymenoptera) on Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae). *Orient. Insects* **2011**, *45*, 275–280. [CrossRef]
- 22. Nagrare, V.S.; Kranthi, S.; Kumar, R.; DharaJothi, B.; Amutha, M.; Deshmukh, A.J.; Bisane, K.D.; Kranthi, K.R. *Compendium of Cotton Mealybug*; Central Institute for Cotton Research: Nagpur, India, 2011; 42p.
- 23. Prasad, Y.G.; Prabhakar, M.; Sreedevi, G.; Thirupathi, M. Spatio-temporal dynamics of the parasitoid, Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae) on mealybug, Phenacoccus solenopsis Tinsley in cotton based cropping systems and associated weed flora. *J. Biol. Control* **2011**, *25*, 198–202.
- 24. Tanwar, R.K.; Jeyakumar, P.; Singh, A.; Jafri, A.; Bambawale, O.M. Survey for cotton mealybug, Phenacoccus solenopsis (Tinsley) and its natural enemies. *J. Environ. Biol.* **2011**, *32*, 381–384.
- 25. Arif, M.I.; Rafiq, M.; Wazir, S.; Mehmood, N.; Ghaffar, A. Studies on cotton mealybug, Phenacoccus solenopsis (Pseudococcidae: Homoptera), and its natural enemies in Punjab, Pakistan. *Int. J. Agric. Biol.* **2012**, *14*, 557–562.
- 26. Çalişkan, A.F.; Hayat, M.; Ulusoy, M.R.; Kaydan, M.B. Parasitoids (Hymenoptera: Encyrtidae) of an invasive mealybug Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) in Turkey. *Turk. J. Entomol.* **2016**, *40*, 133–148. [CrossRef]
- 27. Li, J.F.; Deng, J.; Chen, H.Y.; Yang, L.; Zhou, Z.S.; Jiang, J.J.; Huang, L.F.; Gui, F.R.; Chen, H.S. Investigation on the occurrence of parasitic wasps of Phenacoccus solenopsis Tinsley in Guangxi. *J. South. Agric.* **2020**, *51*, 853–861.
- Torfi, E.T.; Rasekh, A.; Moravvej, S.A.; Mossadegh, M.S.; Rajabpoor, A. Associated Chalcidoidea (Hymenoptera) with the cotton mealybug Phenacoccus solenopsis (Hemiptera: Pseudococcidae) in the southwestern Iran. J. Crop Prot. 2020, 29, 129–139.
- 29. Li, Z.; Yao, T.; Xu, Z.; Meng, L.; Li, B. A new species of Cheiloneurus Westwood (Hymenoptera, Encyrtidae) as a hyperparasitoid of the invasive cotton mealybug, Phenacoccus solenopsis Tinsley, in China. *ZooKeys* **2020**, *974*, 23–29. [CrossRef] [PubMed]
- 30. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; Dewaard, J.R. Biological identifications through DNA barcodes. *Proc. R. Soc. B Boil. Sci.* **2003**, *270*, 313–321. [CrossRef]
- Hebert, P.D.; Ratnasingham, S.; De Waard, J.R. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. B Boil. Sci.* 2003, 270, S96–S99. [CrossRef]
- 32. Venkatesan, T.; Baby, N.L.; Jalali, S.K.; Shylesha, A.N.; Rabindra, R.J. Characterization and identification of Acerophagus papayae Noyes and Schauff (Hymenoptera: Encyrtidae), an introduced parasitoid of papaya mealybug, Paracoccus marginatus Williams and Granara de Willink through DNA barcode. J. Biol. Control 2011, 25, 11–13.
- Zhou, Q.-S.; Xi, Y.-Q.; Yu, F.; Zhang, X.; Li, X.-J.; Liu, C.-L.; Niu, Z.-Q.; Zhu, C.-D.; Qiao, G.-X.; Zhang, Y.-Z. Application of DNA barcoding to the identification of Hymenoptera parasitoids from the soybean aphid (Aphis glycines) in China. *Insect Sci.* 2013, 21, 363–373. [CrossRef]

- Malausa, T.; Delaunay, M.; Fleisch, A.; Groussier-Bout, G.; Warot, S.; Crochard, D.; Guerrieri, E.; Delvare, G.; Pellizzari, G.; Kaydan, M.B.; et al. Investigating Biological Control Agents for Controlling Invasive Populations of the Mealybug Pseudococcus comstocki in France. *PLoS ONE* 2016, *11*, e0157965. [CrossRef] [PubMed]
- 35. Dong, Z.; Liu, S.; Zhang, Z. Efficacy of using DNA barcoding to identify parasitoid wasps of the melon-cotton aphid (Aphis gossypii) in watermelon cropping system. *BioControl* **2018**, *63*, 677–685. [CrossRef]
- Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 1994, 3, 294–299.
- 37. Quicke, D.L.J.; Belshaw, R. Incongruence between Morphological Data Sets: An Example from the Evolution of Endoparasitism Among Parasitic Wasps (Hymenoptera: Braconidae). *Syst. Biol.* **1999**, *48*, 436–454. [CrossRef]
- 38. Campbell, B.C.; Steffen-Campbell, J.D.; Werren, J.H. Phylogeny of the Nasonia species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Mol. Biol.* **1994**, *2*, 225–237. [CrossRef]
- Gillespie, J.J.; Munro, J.B.; Heraty, J.M.; Yoder, M.J.; Owen, A.K.; Carmichael, A.E. A Secondary Structural Model of the 28S rRNA Expansion Segments D2 and D3 for Chalcidoid Wasps (Hymenoptera: Chalcidoidea). *Mol. Biol. Evol.* 2005, 22, 1593–1608. [CrossRef]
- 40. Belshaw, R.; Quicke, D.L. A Molecular Phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Mol. Phylogene. Evol.* **1997**, *7*, 281–293. [CrossRef]
- 41. Belshaw, R.; Lopez-Vaamonde, C.; Degerli, N.; Quicke, D.L. Paraphyletic taxa and taxonomic chaining: Evaluating the classification of braconine wasps (Hymenoptera: Braconidae) using 28S D2–3 rDNA sequences and morphological characters. *Biol. J. Linn. Soc.* **2001**, *73*, 411–424. [CrossRef]
- 42. Chesters, U.; Wang, Y.; Yu, F.; Bai, M.; Zhang, T.-X.; Hu, H.-Y.; Zhu, C.-D.; Li, C.-D.; Zhang, Y.-Z. The Integrative Taxonomic Approach Reveals Host Specific Species in an Encyrtid Parasitoid Species Complex. *PLoS ONE* **2012**, *7*, e37655. [CrossRef]
- 43. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef] [PubMed]
- 44. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef]
- 45. Puillandre, N.; Lambert, A.; Brouillet, S.; Achaz, G. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* **2011**, *21*, 1864–1877. [CrossRef] [PubMed]
- 46. Zhang, J.; Kapli, P.; Pavlidis, P.; Stamatakis, A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **2013**, *29*, 2869–2876. [CrossRef]
- 47. Peters, R.S.; Krogmann, L.; Mayer, C.; Donath, A.; Gunkel, S.; Meusemann, K.; Kozlov, A.; Podsiadlowski, L.; Petersen, M.; Lanfear, R.; et al. Evolutionary History of the Hymenoptera. *Curr. Biol.* **2017**, *27*, 1013–1018. [CrossRef]
- 48. Ruan, Y.Y.; Wang, Z.H.; Huang, J. A new record genus and species of Aphelinidae (Hymnoptera, Chalcidoidea) from China. *Acta Zootaxon. Sin.* **2012**, *37*, 456–459.
- 49. Suroshe, S.S.; Gautam, R.D.; Fand, B.B. Natural enemy complex associated with the mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) infesting different host plants in India. *J. Biol. Control* **2013**, *27*, 204–210.
- 50. Chen, H.Y.; Cao, R.X.; Xu, Z.F. First record of Aenasius bambawalei Hayat (Hymenoptera: Encyrtidae), a parasitoid of the mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) from China. *J. Environ. Entomol.* **2010**, *32*, 280–282.
- 51. Yu, H.B.; Liang, W.S.; Fang, T.S.; Pan, Z.P. Investigation of damage and its natural enemies of Phenacoccus solenopsis Tinsley on garden plants in Guangdong Province. *J. Environ. Entomol.* **2015**, *37*, 1109–1112.
- 52. Spodek, M.; Ben-Dov, Y.; Mondaca, L.; Protasov, A.; Erel, E.; Mendel, Z. The cotton mealybug, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) in Israel: Pest status, host plants and natural enemies. *Phytoparasitica* **2018**, *46*, 45–55. [CrossRef]
- Peronti, A.L.B.G.; Martinelli, N.M.; Alexandrino, J.G.; Júnior, A.L.M.; Penteado-Dias, A.M.; Almeida, L.M. Natural Enemies Associated with Maconellicoccus hirsutus (Hemiptera: Pseudococcidae) in the State of São Paulo, Brazil. *Fla. Èntomol.* 2016, 99, 21–25. [CrossRef]
- 54. Noyes, J.S.; Hayat, M. A review of the genera of Indo-Pacific Encyrtidae (Hymenoptera: Chalcidoidea). *Bull. Br. Mus. Nat. Hist. Entomol. Ser.* **1984**, *48*, 131–395.
- 55. Xu, Z.H.; Huang, J. Chinese Fauna of Parasitic Wasps on Scale Insects; Shanghai Scientific & Technical Publishers: Shanghai, China, 2004; 524p.
- 56. Zhang, Y.Z.; Huang, D.W. A Review and an Illustrated Key to Genera of Encyrtidae (Hymenoptera: Chalcidoidea) from China; Science Press: Beijing, China, 2004; 95p.
- 57. Hayat, M. *Indian Encyrtidae (Hymenoptera: Chalcidoidea)*; Department of Zoology, Aligarh Muslim University: Aligarh, India, 2006; 496p.
- 58. Wang, Z.H.; Huang, J.; Xu, Z.H. Chinese species of the genus Prochiloneurus Silvestri with description of a new species (Hymenoptera: Encyrtidae). *Zoolog. Syst.* 2014, *39*, 424–432.
- 59. Sureshan, P.; Narendran, T. Description of a new species of Ophelosia Riley with synonymic and taxonomic notes on Indian Eunotinae (Hymenoptera: Chalcidoidea: Pteromalidae). *Zoos Print J.* **2005**, *20*, 1854–1855. [CrossRef]
- 60. Pillai, K.G.; Krishnamoorthy, A.; Gangavisalakshy, P.N. First report of hyperparasitoids of Anagyrus dactylopii (Howard) from India. J. Biol. Control 2009, 23, 193–194.

- 61. Noyes, J.S. Universal Chalcidoidea Database. Available online: http://www.nhm.ac.uk/research-curation/projects/chalcidoids (accessed on 30 August 2020).
- 62. Guerrierri, E.; Ghahari, H. New records, descriptions and notes on Encyrtidae (Hymenoptera: Chalcidoidea) from Iran. *Zootaxa* **2018**, 4444, 316–326. [CrossRef]
- 63. Joshi, S.; Rameshkumar, A.; Mohanraj, P. New host-parasitoid associations for some coccids (Hemiptera: Coccoidea) from India. J. *Entomol. Res.* **2017**, *41*, 177. [CrossRef]