JHR 99: 1045–1067 (2026)
doi: 10.3897/jhr.99.202665
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Integrative species delimitation and preliminary study of host specificity of encyrtid parasitoids associated with psyllids in China

Boyu Zou[1,2], Douglas Chesters[1,2], Runze Yu[1,3], Huanji Liu[1,2], Hongliang Li[1], Hui Xiao[1,2], Chaodong Zhu[1,2], Yanzhou Zhang[1,2]

1 *State Key Laboratory of Animal Biodiversity Conservation and Integrated Pest Management, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China* **2** *College of Biological Science, University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China* **3** *College of Life Science and Technology, Xinjiang University, Urumqi, Xinjiang 830017, China*

Corresponding author: Yanzhou Zhang (zhangyz@ioz.ac.cn)

Academic editor: Zachary Lahey | Received 4 June 2026 | Accepted 27 July 2026 | Published 25 September 2026

https://zoobank.org/4D2D59F9-CAF2-44AE-B41D-8DCF184FDFBC

Citation: Zou B, Chesters D, Yu R, Liu H, Li H, Xiao H, Zhu C, Zhang Y (2026) Integrative species delimitation and preliminary study of host specificity of encyrtid parasitoids associated with psyllids in China. Journal of Hymenoptera Research 99: 1045–1067. https://doi.org/10.3897/jhr.99.202665

Abstract

In the family Encyrtidae (Hymenoptera, Chalcidoidea), many species attack psyllids (Hemiptera, Psylloidea) and, thus, are instrumental in controlling populations of those hosts. However, successful biological control requires accurate identification of encyrtid parasitoids and a clear picture of specificity in host-parasitoid interactions. Taxonomic classification, species delimitation and host affiliation remain challenging for researchers due to their the small size of parasitoid wasps, morphological complexity, and the prevalence of cryptic species in the family. In the present paper, we conducted a DNA barcoding study of encyrtid parasitoids of psyllids collected from many provinces throughout China over the past two decades. We analyzed host specificity within an integrative taxonomic framework that combined both morphological data and DNA barcodes. Multiple species-delimitation analyses (ABGD, ASAP, GMYC and bPTP) were used, with a range of 32 to 47 Molecular Operational Taxonomic Units (MOTUs) returned, indicating cryptic diversity among morphological forms. By integrating morphological evidence with molecular species-delimitation results, we recognized 32 species in our samples. Host specificity analysis indicated more than 80% of parasitoid species were strictly monophagous, each parasitizing no more than a single psyllid species. This study provides a preliminary clarification of the species status of Chinese encyrtids parasitoids of psyllids and establishes a DNA barcode database covering their diversity.

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Keywords
Psylloidea, Encyrtidae, Integrative taxonomy, Host affiliation, Parasitoid, Biological control

Introduction

Psyllids (Hemiptera: Psylloidea), constitute a globally distributed group of sap-sucking insects of over 4,000 described species (Burckhardt et al. 2021). They exhibit a high degree of host plant specificity and primarily associate with trees and shrubs of various families, including Rutaceae, Rosaceae, and Fabaceae (Hodkinson 1974, 1980; Burckhardt and Ouvrard 2012). Many psyllids are economically important pests and pose a continuous threat to fruit and other horticultural industries (Hodkinson 1974; Li 2011; Mauck et al. 2024). The family Encyrtidae represents one of the predominant natural enemies of psyllids (Noyes and Hayat 1984; Noyes and Hanson 1996). According to the Universal Chalcidoidea Database (UCD Community (2023), https://ucd.chalcid.org), around 19 genera are found to be associated with psyllids. Encyrtid parasitoids of psyllids are predominantly specialist endoparasitoids of psyllid nymphs (Noyes and Hanson 1996). Many encyrtid parasitoids of psyllid species have been employed in biological control programs worldwide. *Psyllaephagus pilosus* Noyes, 1988 from Australia was introduced and released in California and European countries to control *Ctenarytaina eucalypti* Maskell, 1890 (Dahlsten et al. 1998; Chauzat et al. 2002). *Psyllaephagus bliteus* Riek, 1962 was introduced and released in California to control *Glycaspis brimblecombei* Moore, 1964 (Daane et al. 2005). *Diaphorencyrtus aligarhensis* Shafee, Alam & Agarwal, 1975 was introduced and released in California to control *Diaphorina citri* Kuwayama, 1908 (Bistline-East et al. 2015; Milosavljević et al. 2022).

Accurate species identification is a prerequisite for biocontrol research and application (Gordh and Beardsley 1999). Due to the small body size of encyrtid parasitoids associated with psyllids (usually 0.5–2.0 mm), high-quality slide- and card-mounted specimens, high resolution images, informative keys and thorough taxonomic descriptions are essential for species identification (Noyes 1982; Wang et al. 2016; McClelland et al. 2023). Challenge in species discrimination is compounded by the prevalence of cryptic species (Smith et al. 2008; Chesters et al. 2012; Qin et al. 2018). Over the past two decades, DNA barcoding has become a powerful, objective and widely used method for species delimitation (Hebert et al. 2003). In hymenopteran parasitoids, DNA barcoding has been frequently applied to identify species (Smith et al. 2008, 2013; Stahlhut et al. 2013; Zhou et al. 2014; Fernandez-Triana et al. 2023; Xu et al. 2023) and, in the family Encyrtidae, DNA barcoding has been similarly applied in many genera associated with scale insect hosts (Chesters et al. 2012; Qin et al. 2018; Rudoy et al. 2022). However, molecular taxonomic studies of encyrtid parasitoids of psyllids is rare except that of Fromont et al. (2017), Hall et al. (2017), McClelland et al. (2023) and McClelland et al. (2025).

Host specificity is key to the habits and distribution of parasitoids (Poulin and Keeney 2008) because it reflects the range of host species that a parasitoid can successfully exploit. The distributions of highly specialized parasitoids may therefore be more closely constrained by the availability of suitable hosts, whereas parasitoids with broader host ranges may occur across a wider range of host communities and habitats (Godfray 1994; Hawkins 1994). Host specificity has attracted considerable interest from ecologists and evolutionary biologists (Godfray 1994; Dyer et al. 2007; Guilhaumon et al. 2012). Many studies have determined that a single, generalist morphospecies might actually comprise multiple host specific species (Heraty et al. 2007; Smith et al. 2008; Li et al. 2010; Nicholls et al. 2010; Hrček and Godfray 2015; Qin et al. 2018). Noyes and Hanson (1996) indicated that some species of *Psyllaephagus* Ashmead appear to be host specific. Field observations suggest that other species exhibit narrow host ranges, such as *D. aligarhensis* (Qureshi et al. 2009) and *Trechnites insidiosus* (Tougeron et al. 2021). A DNA-based food-web analysis of eight *Eucalyptus*-feeding psyllid species revealed high host specificity among parasitoid lineages (Hall et al. 2017). To date, specific host specificity of encyrtid parasitoids associated with psyllids remains poorly understood.

In the present study, we present a preliminary DNA barcoding analysis of encyrtid parasitoids of psyllids collected across China and subsequently analyze patterns of host specificity using an integrative taxonomic approach that combines detailed morphological examination with molecular data. This study aims to: (1) delineate species of Chinese encyrtid parasitoids associated with psyllids using an integrative taxonomic approach (morphology, molecules and host records); (2) establish a reliable and publicly accessible DNA barcode reference library for this group in China; and (3) provide a preliminary assessment of host specificity patterns via a host–parasitoid interaction network.

Materials and methods

Specimen sampling

We examined more than 2,000 parasitoid wasps associated with psyllids sourced from both historical museum collections and extensive fieldwork that covered 22 provincial-level administrative divisions of China. To rear parasitoids, plant materials (twigs, leaves, and bark) infested with late-stage nymphs of psyllids were collected and maintained in glass vials or fine-mesh nylon bags. These rearing efforts yielded approximately 1,000 encyrtid wasps from 400 psyllid-rearing batches. Of all specimens examined, 263 representative individuals were selected for sequencing (Suppl. material 1). When multiple psyllid species infested the same host plant, each species was isolated and reared separately for at least two months to ensure complete parasitoid emergence. The identified host psyllids included gall-forming and non-galling, foliage-dwelling taxa representing four families–Aphalaridae, Carsidaridae, Psyllidae, and Triozidae; detailed

host identities and feeding habits are provided in Suppl. material 1. Upon emergence, parasitoids were preserved in 95% ethanol for molecular analysis.

For morphological identification of species, we used the following keys: Trjapitzin (1989), Noyes and Hanson (1996), Hayat (2006), Zhang and Huang (2004) and Zou et al. (2023). All specimens used for molecular analyses and listed in Suppl. material 1 are deposited in the Institute of Zoology, Chinese Academy of Sciences.

DNA extraction, PCR amplification and sequencing

Approximately 67% of the encyrtid parasitoids examined were used in DNA barcoding study. Total genomic DNA was extracted from each individual using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocols. The COI gene was amplified and sequenced using the universal DNA barcoding primers LCO1490 and HCO2198 (Folmer et al. 1994). For specimens that proved difficult to amplify, the primer pair FWPTF (Li et al. 2010) and LepR1 (Hebert et al. 2004) was used. Sequences of the 28S-D2 region (D2 expansion segment of 28S rDNA) were generated using the primer pairs D2-3549 and D2-4068 (Campbell et al. 1993), or D2-3566 and D2-4057 (Gillespie et al. 2005). Reactions (30 μl) consisted of 3.3 μl of template DNA (not quantified), 9 μl of reaction buffer, 4 μl of dNTP mixture (2.5 mM each), 0.5 μM of each primer and 1.25 units of ExTaq polymerase. Following Deng et al. (2012) and Zhang et al. (2011), amplification comprised an initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 50 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. All of the sequences were queried in the National Center for Biotechnology Information (NCBI) nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with default parameters.

Barcoding analysis

DNA sequences were checked and assembled with BioEdit (Hall 1999). In MEGA X (Kumar et al. 2018), consensus sequences were translated with the mitochondrial invertebrate genetic code to check for stop codons, and genetic interspecific and intraspecific distances were calculated with the Kimura two-parameter (K2P) model (Kimura 1980). Both distance-based (ASAP and ABGD) and tree-based (GMYC and bPTP) methods were used to evaluate barcoding success rates in the COI data set.

Distance based methods

The ABGD analysis was performed online (https://bioinfo.mnhn.fr/abi/public/abgd/) (Puillandre et al. 2012) under Jukes-Cantor (JC69; Jukes and Cantor 1969), Kimura 2-parameter (K2P), and uncorrected distance p-distance (Nei and Kumar 2000), with relative gap width (X = 1.0). The range of prior intraspecific divergence from 0.001 to

0.1 was recorded with 20 steps. ASAP was implemented using the same three models (Simple, K2P, JC69). Threshold genetic distances were dynamically selected, and the optimal partition was determined by minimizing the ASAP score, which balances over-splitting and under-splitting errors. The number of proposed species groups was evaluated across varying thresholds. The aligned sequences were submitted online (https://bioinfo.mnhn.fr/abi/public/asap/) (Puillandre et al. 2021).

Tree based methods

For creation of the Bayesian time-calibrated tree, a partitioned relaxed molecular clock model was implemented in BEAST2. Substitution models for each partition were selected using jModelTest (Posada 2008) (COI: GTR+I+G). A Yule speciation prior (Yule 1925) and an uncorrelated lognormal clock model were applied (Drummond et al. 2006). Two independent MCMC chains were run for 50 million generations, sampling every 5,000 steps. Convergence and effective sample sizes (ESS > 200) were verified in Tracer v1.7.2 (Rambaut et al. 2018). Maximum clade credibility trees were summarized in TreeAnnotator v2.6.6 (Helfrich et al. 2018) after discarding the first 10% as burn-in. NJ trees based on K2P distance model were built in MEGA X using 500 bootstrap replicates.

GMYC analyses were performed in the R package splits (Ezard et al. 2009; Fujisawa and Barraclough 2013). Single- and multiple-threshold models were fitted to the ultrametric chronograms generated in BEAST (Pons et al. 2006; Monaghan et al. 2009). Likelihood ratio tests (LRTs) compared the fit of coalescent vs. speciation models. Threshold transitions and 95% confidence intervals were estimated via parametric bootstrapping (1,000 replicates).

For the bPTP analysis we first constructed a Bayesian tree in PhyloSuite v1.1.2 (Zhang et al. 2020), then imported it into the online URL of bPTP (https://species.h-its.org/ptp/) (Zhang et al. 2013) and set the number of MCMC iterations to 5,000,000. The first 25% was discarded as burn-in and the other parameters were default for the analysis.

Host specificity analysis

To accurately assess host utilization by parasitoids, the food web was reconstructed using species delimitation results combining the morphological and DNA barcoding analyses. Network-level metrics, including connectance and the specialization index H2' (Blüthgen et al. 2006), were calculated using the bipartite package (v2.18) (Dormann et al. 2009) in R (v4.4.2). Species-level specialization indices were also calculated: d' (degree of host monopolization; Blüthgen et al. 2006) and Paired Difference Index (PDI; reliance on primary host; Poisot et al. 2010), and Species Specificity Index (SSI; evenness of interaction frequencies across hosts; Julliard et al. 2006). All analyses were performed using the bipartite (v2.18) (Dormann et al. 2009) and ESM (Poisot et al. 2012) packages in R (v4.4.2).

Results

Sample collection and morphological diversity

Morphological examination placed these specimens within 4 genera: *Diaphorencyrtus* Hayat, 1981, *Psyllaephagus* Ashmead, 1900 (the most abundant genus, more than 85% of specimens), *Trechnites* Thomson 1876, and *Prionomitus* Mayr, 1876. Preliminary sorting resulted in the recognition of 29 distinct morphospecies, of which *Psyllaephagus* contributed 24 species, while the remaining four were distributed among *Prionomitus* (2 species), *Trechnites* (2 species), and *Diaphorencyrtus* (1 species).

Sequence variation

A total of 212 COI sequences representing 28 morphospecies in 3 genera were used for barcode analysis, with 209 newly generated from collected individual psyllids and three downloaded from NCBI (GenBank: MZ438311, MZ436006 and OP787025). We were not able to obtain COI sequences from *Psyllaephagus calligonicola* or *Diaphorencyrtus aligarhensis*. The length of all sequenced fragments was 540 bp after edge trimming, with 288 conserved sites, 246 variable sites, and 250 parsimony-informative sites. No insertions, deletions, or stop codons were noted in the sequences. All COI sequences had a bias toward low GC content (A = 26.3%, T = 45.6%, C = 13.5%, and G = 14.7%).

The mean intraspecific divergence was 0.012 (ranging from 0.00 to 0.0879), the mean interspecific divergence was 0.1492 (ranging from 0.0537 to 0.2276). The interspecies divergence within genera varied from 4.88% to 13.58%: *Trechnites* (mean = 7.55%), *Prionomitus* (mean = 4.88%) and *Psyllaephagus* (mean = 13.58%) (detailed values of each genus are provided in Suppl. material 2). For intraspecific differentiation, 1) the lowest divergence occurred between *Prionomitus tiliaris*, *Trechnites* sp 1, *Psyllaephagus femoralis*, *Psyllaephagus* sp 2, *Psyllaephagus* sp 4 (0.00) and *Psyllaephagus obliquus* (0.0004); 2) the highest diversity was observed in *Psyllaephagus caillardiae* (0.0879), followed by *Psyllaephagus arenarius* (0.0471), suggesting higher population-level variability; 3) most groups (e.g., *Psyllaephagus nartshukae*, *Psyllaephagus colposceniae*) exhibited moderate divergence (0.0012–0.0318). For interspecific differentiation, 1) the lowest divergence occurred between *Psyllaephagus* sp 2 and *Psyllaephagus* sp 3 (0.0537), indicating potential close relatedness; 2) the highest divergence was observed between *Psyllaephagus* sp 2 and *Trechnites* sp 1 (0.2276), supporting their distinct species status (see Fig. 1 and Suppl. material 2). The length of the 255 nuclear 28S sequences ranged from 519 bp in *Psyllaephagus stenopsyllae* to 481 bp in *Prionomitus tiliaris*. The mean inter- and intraspecific divergences were 4.44% and 0.406%, respectively.

Molecular species delimitation

In the ABGD analysis, K2P and JC69 models yielded identical results: initial partitioning suggested 32 groups at P = 0.02976, while recursive partitioning increased group

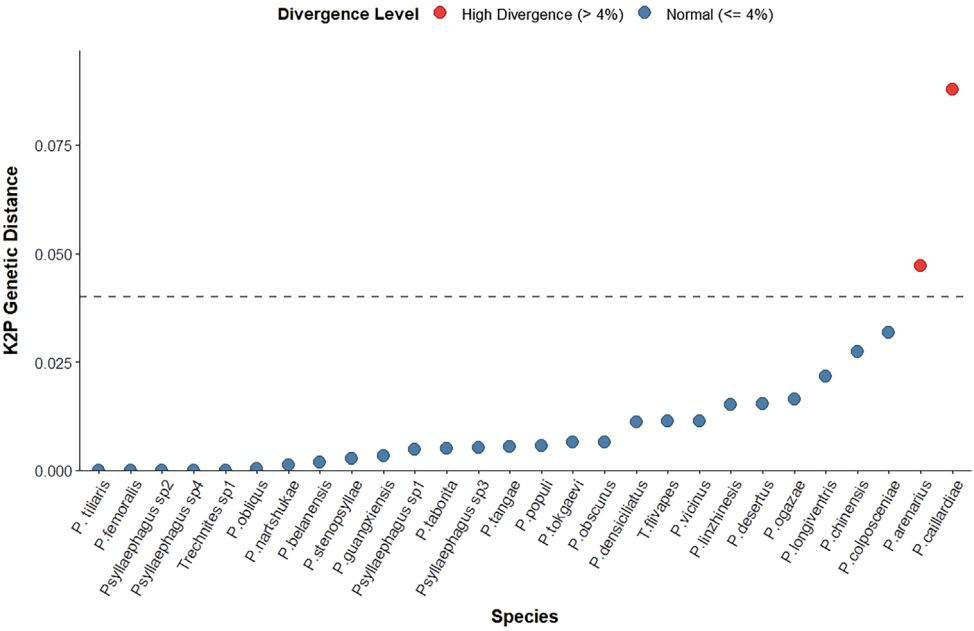

Figure 1. Maximum intraspecific Kimura two-parameter (K2P) distances among 212 COI sequences representing 28 morphospecies of encyrtid parasitoids associated with psyllids in China. Red points indicate divergence > 4%, blue points indicate divergence ≤ 4%, and the dashed line marks the 4% reference threshold.

numbers to 33–45 at intermediate P values, stabilizing at 71 groups for P ≤ 0.002069. The simple distance model produced fewer groups initially (32–40) but stabilized at 17 groups under recursive partitioning for P ≤ 0.00336. At a priori genetic distance thresholds of 0.02967 and 0.04833, ABGD strongly supported the presence of 32 genetically distinct partitions in the COI data set (see Suppl. material 4: table S4-1). The number of partitions produced by ABGD was in accord with morphological identifications. ABGD strongly supported the presence of 32 genetically distinct partitions in the COI data set. Among these morphological species, *P. colposceniae*, *P. chinensis*, *P. caillardiae*, and *P. arenarius* were each distinguished into two groups (Fig. 2). The ASAP analysis produced 30 MOTUs under JC69, the simple models (ASAP scores 6.00, 6.50) and K2P model (ASAP score 7.00) (Suppl. material 4: table S4-2). In contrast to the ABGD analysis, the ASAP analysis with the K2P model revealed more MOTUs within three morphospecies, splitting *P. colposceniae* into three and *P. longiventris* into two. Additionally, the JC69 and simple models grouped *P. desertus* and *P. caillardiae* (from Changji, Xinjiang) as a single MOTU (Fig. 2).

A total of 113 COI haplotypes and 81 nuclear 28S haplotypes were used to construct the NJ trees. Based on tree topology and branch support, the COI haplotypes were resolved into 32 distinct clades corresponding to the 32 consensus MOTUs, whereas the 28S haplotypes were resolved into 34 clades (Suppl. material 5, 6).

The GMYC single-threshold analysis identified 38 species (95% CI: 32–43) and 39 entities (95% CI: 33–44). The likelihood ratio test was highly significant (LR =

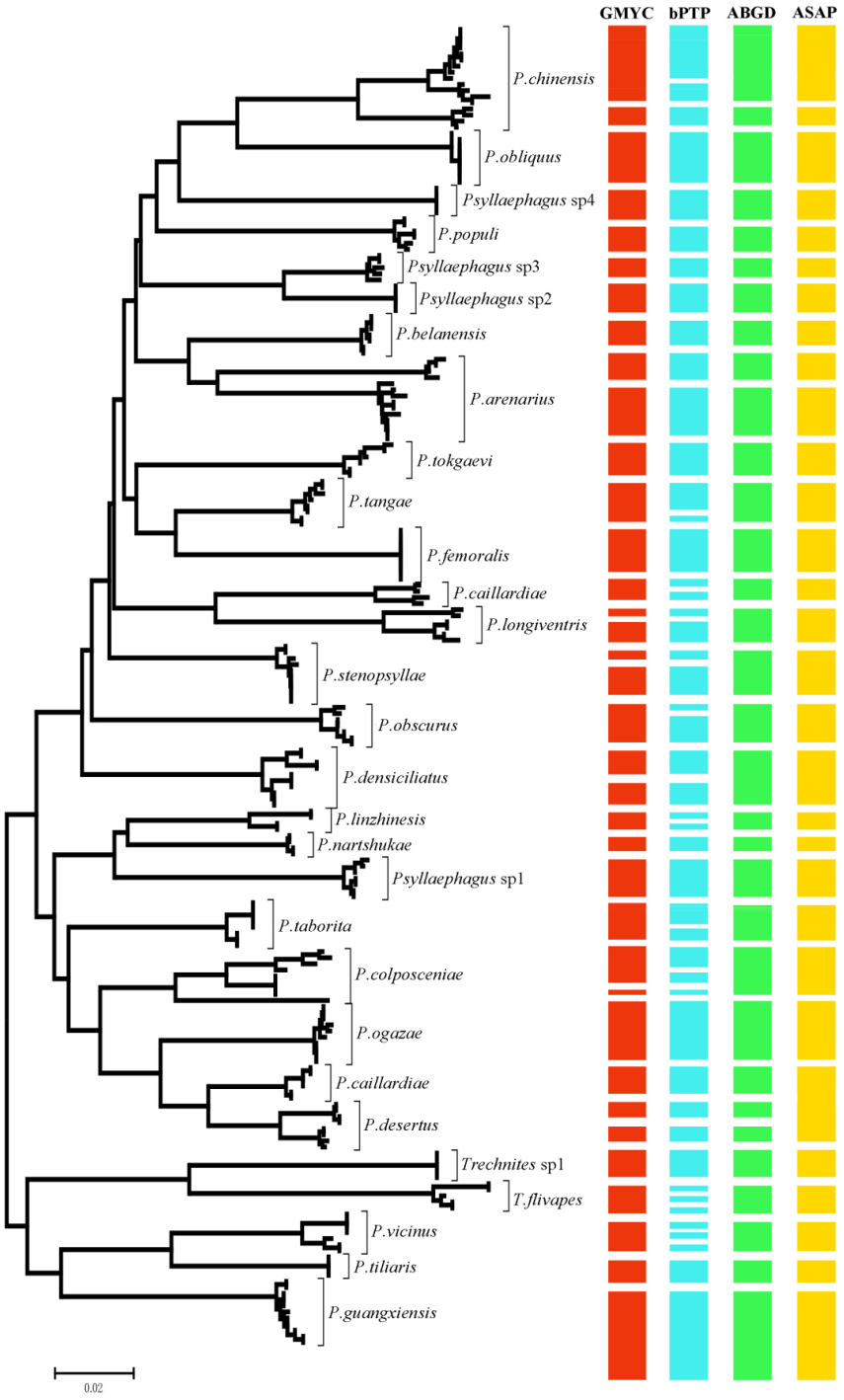

Figure 2. Neighbor Joining (NJ) tree of 216 COI sequences, including morphological analysis and results of four different species delimitation methods (Color bars indicate the results of ABGD, ASAP, GMYC, bPTP).

77.67, P < 0.001), rejecting the null coalescent model. Threshold time was -0.0092 relative to tree root. Consistent with distance-based methods, GMYC supported delimitation of *P. chinensis*, *P. arenarius*, *P. colposceniae*, and *P. caillardiae* as distinct MOTUs, while further splitting *P. desertus*, *P. densiciliatus*, and *P. stenopsyllae* (Fig. 2). The bPTP analysis showed an acceptance rate of 34.7%, with 249,749 merge events and 250,251 split events. The estimated number of species ranged broadly between 42 and 68, with a mean of 53.6.

Maximum Likelihood analyses of our dataset distinguished 49 MOTUs; and Bayesian analyses distinguished 47 MOTU (Fig. 2). Although the tree-based bPTP method yielded a significantly higher count of MOTUs (47–49), likely due to oversplitting of intraspecific population structure, the distance-based ABGD and tree-based GMYC analyses showed strong congruence in revealing cryptic diversity. Both methods consistently supported the splitting of four morphospecies (*P. colposceniae*, *P. chinensis*, *P. caillardiae*, and *P. arenarius*) into distinct lineages. Consequently, adopting a conservative consensus approach across multiple delimitation methods, we recognized a total of 32 MOTUs from the 29 initial morphospecies in this study (Fig. 2).

Host specificity of encyrtid parasitoids of psyllids in China

To assess host use pattern, a tanglegram showing host–parasitoid interactions was established for those species with host records (Fig. 3). The network comprised 21 host

Table 1. Measuring host specificity using molecular species-level indices in parasitoid–host network.

Parasitoids	d'	SSI	PDI
Trechnites sp 1	1	1	1
Trechnites flavipes	1	1	1
Psyllaephagus tokgaevi	0.8847472	1	1
Prionomitus tiliaris	1	1	1
Psyllaephagus tangae	1	1	1
Psyllaephagus stenopsyllae	0.693163	0.5608773	0.93
Psyllaephagus populi	1	1	1
Psyllaephagus ogazae	0.5925446	0.814862	0.9875
Psyllaephagus obliquus	1	1	1
Psyllaephagus longiventris	0.7102146	1	1
Psyllaephagus linzhiensis	1	1	1
Psyllaephagus guangxiensis	0.6931138	0.5477226	0.9
Psyllaephagus femoralis	0.746215	1	1
Psyllaephagus desertus	0.7660403	1	1
Psyllaephagus chinensis	1	0.7091957	0.96875
Psyllaephagus colposceniae	0.6609728	1	1
Psyllaephagus caillardiae	1	1	1
Psyllaephagus belanensis	1	1	1
Psyllaephagus arenarius	1	1	1
Psyllaephagus arenarius nr1	1	1	1
Psyllaephagus caillardiae nr1	0.5316736	1	1
Psyllaephagus colposceniae nr1	0.5103994	1	1
Psyllaephagus chinensis nr1	1	1	1

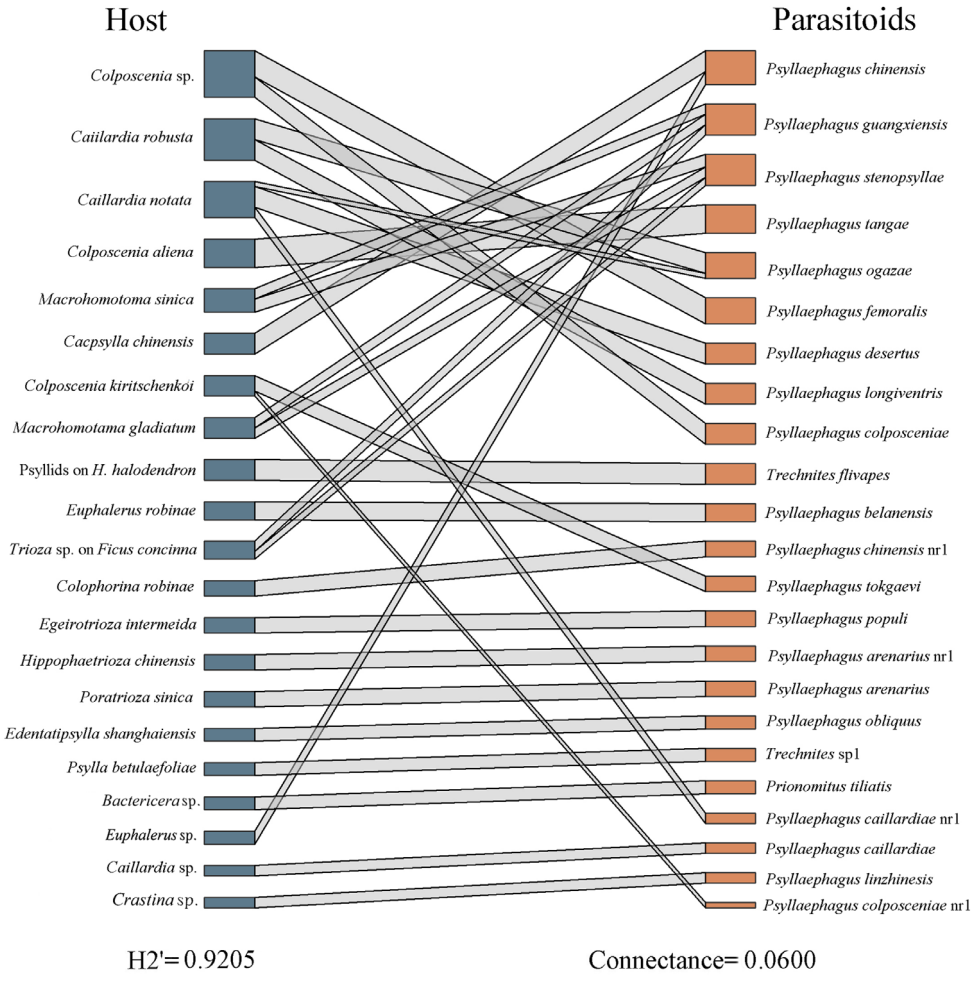

Figure 3. Encyrtid-Psyllid interaction networks based on MOTU.

species and 23 parasitoid species. Nine of the 32 MOTUs were excluded from the network because host records were unavailable. The resulting bipartite network was sparse but highly structured, with 19 parasitoid species utilizing only a single host species; two parasitoid species utilizing two host species, and two parasitoid species utilizing three host species. At the network level, connectance was low (0.06) and the specialization index was exceptionally high (H2' = 0.9205), indicating strong overall specialization. Species-level metrics reinforced this finding: over half of the parasitoid species showed extreme host specificity (d' = 1.0). The few apparent generalists—*P. stenopsyllae* and *P. guangxiensis*—each attacked three host species and had relatively low SSI values (<0.6). However, their high PDI values (>0.9) revealed that even these species strongly preferred a single primary host. Additionally, we found seven psyllid species were parasitized by two or three parasitoid species.

Discussion

Efficacy of DNA barcoding for species identification

DNA barcoding analyses demonstrated that COI sequences effectively discriminate all encyrtid morphospecies. The presence of a clear barcoding gap and high bootstrap support for species-level clades in phylogenetic trees (Suppl. material 5) indicate that COI possesses sufficient variation to distinguish most species in this group. These findings are consistent with successful COI barcoding applications in other encyrtid parasitoid wasps (Zhang et al. 2011; Chesters et al. 2012; Wang et al. 2016; Qin et al. 2018) and parasitoid wasp families more broadly (e.g., Smith et al. 2008, 2013; Xu et al. 2023). Notably, even between closely related species (e.g., *Psyllaephagus* sp 2 and *Psyllaephagus* sp 3), COI exhibited an average interspecific genetic distance of 5.37%, comparable to values reported for the *Encyrtus sasakii* complex (Chesters et al. 2012).

In the present study, the minimum interspecific threshold observed for COI (approximately 5.37%) may be attributable to its slower evolutionary rate compared to other mitochondrial markers. This interpretation is supported by recent mitogenomic analyses of Encyrtidae, which revealed that COI exhibits the lowest substitution rate among all 13 mitochondrial protein-coding genes (Zhang et al. 2024). Using mitochondrial Cytochrome b (Cytb), Hall et al. (2017) found the three *Psyllaephagus* morphospecies reared from eight *Eucalyptus*-feeding psyllid species might contain nine putative cryptic species, suggesting that Cytb, with its relatively higher interspecific genetic distance threshold (approximately 8.4%), may resolve additional cryptic species. The available evidence suggests that COI may evolve relatively slowly in encyrtid parasitoids associated with psyllids, although comparisons with a broader range of mitochondrial genes are needed to confirm this inference.

Although the 28S-D2 region is known to be more evolutionarily conserved than COI (Heraty et al. 2004), it remains a valuable complementary tool for species confirmation in Encyrtidae (Zhang et al. 2011; Wang et al. 2016; Qin et al. 2018). Our results support the utility of this marker for distinguishing encyrtid parasitoid species associated with psyllids, as it exhibited a clear barcode gap in interspecific genetic distances (Suppl. material 3: table S3-1, S3-2). Notably, 28S-D2 distinguished between closely related species (e.g., *P. chinensis* and *P. obliquus*) based on fixed diagnostic nucleotide substitutions, demonstrating that even a conserved nuclear marker can provide reliable species-level resolution when diagnostic sites are present. The presence of such diagnostic characters enhances the reliability of molecular identification and provides a stable reference for future taxonomic and biological control studies.

Cryptic species diversity

The advent of molecular taxonomy has greatly facilitated the discovery of cryptic diversity (Bickford et al. 2007). Numerous studies have demonstrated that cryptic species are prevalent in parasitoid wasps (Heraty et al. 2007; Smith et al. 2008; Nicholls et al.

2010), with some morphospecies in Encyrtidae comprising multiple MOTUs (Wang et al. 2016). However, the extent of cryptic diversity and the contribution of molecular approaches vary among taxonomic groups. Evidence from large-scale taxonomic surveys increasingly supports high concordance between morphology-based and molecular-based species delimitation in lineages with unambiguous diagnostic characters (Jablonski and Finarelli 2009; Chen et al. 2011; Pentinsaari et al. 2014; Toro-Delgado et al. 2022). In such morphologically challenging groups associated with psyllids, we found some characters, such as the shape of the oblique portion of clava and the coloration of coxae, provide complementary evidence to molecular data, helping to resolve species boundaries and improve taxonomic resolution.

Among Chinese encyrtid parasitoids associated with psyllids, we observed a high degree of concordance between morphological and molecular boundaries, with cryptic lineages detected in only several morphospecies (e.g. *P. chinensis* and *P. arenarius*). The congruence between molecular delimitations and morphological classifications demonstrates that morphological characters provide reliable and practical means for species identification in this group, reducing the likelihood that morphologically cryptic lineages have been overlooked.

Host specificity of encyrtid parasitoids associated with psyllids

The degree of host specificity of encyrtid parasitoids of psyllids is not known with any certainty, but it appears that host specificity is presumed to be high (Noyes and Hanson 1996); however, this critical biological trait has been rigorously tested in only a few cases. By integrating molecular delimitation with extensive geographic sampling and network analysis, we provide a framework for host specificity of these parasitoids. Our result revealed that 19 species with known host records are restricted to a single host species, and two species are associated with three host species. Our findings indicated a high level host specificity exists in encyrtid parasitoids associated with psyllids. Even a few species expand beyond a single host species, but in these cases the hosts are usually closely related species. This result is consistent with other studies of encyrtid parasitoids associated with scale insects (Zhang et al. 2011; Chesters et al. 2012; Wang et al. 2016; Qin et al. 2018). As endoparasitoids, they must overcome specific host defenses (Salt 1968), whereby many physical and chemical factors may become increasingly refined in the interactions with the hosts (Vinson 1976).

The introduction of molecular data has refined the understanding of parasitoid host specialization (Smith et al. 2006, 2008, 2013; Kaartinen et al. 2010). Patterns of host use in *Psyllaephagus* often reveal high specificity, even within species that exploit multiple host species (Fromont et al. 2017; Hall et al. 2017). Our findings for these parasitoids align with this trend: while monophagy is the predominant pattern (H2'=0.9205), several species, such as *P. stenopsyllae* and *P. guangxiensis*, were found to utilize multiple hosts. However, the high Paired Difference Index (PDI ≥ 0.9) for these species indicates a strong reliance on a single primary host. This outcome is consistent with patterns observed in encyrtid parasitoids of scale insects (Zhang et al. 2011; Qin et al. 2018).

Collectively, these insights suggest that host-parasitoid associations may be tighter than morphological assessments imply. This has significant implications for biological control: while high specialization minimizes non-target risks, it necessitates precise host-parasitoid matching to avoid control failure in dynamic environments.

Conclusion

By combining morphological examination with COI and 28S rDNA sequence analysis, we successfully delimited 32 MOTUs from 29 initial morphospecies, uncovering cryptic diversity within four species. Barcodes combined with morphology-based taxonomy can accelerate the identification of morphologically cryptic species within species complexes. The host-parasitoid network analysis revealed an exceptional degree of specialization (H2'=0.9205), with over 80% of the species exhibiting monophagy. This high host specificity level likely reflects evolutionary constraints imposed by the narrow host ranges of psyllids themselves. However, our current sampling is insufficient to robustly assess how different molecular species-delimitation methods affect the results. We will continue sampling in future studies to investigate this question more thoroughly. Together, the DNA barcode reference library and assessment of parasitoid host specialization provide a foundation for the accurate identification and selection of effective, host-specific natural enemies, thereby improving the efficacy and reducing the non-target risks of future psyllid biocontrol programs in China.

Acknowledgements

We would like to thank Dr. Hong-Ying Hu (Xinjiang University), for providing valuable specimens and information, and to Mr. Shu-Li Zhang in field collection. We thank Dr. Alana McClelland (The University of Adelaide, South Australia, Australia) and another anonymous reviewer for their valuable comments.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

Funding

This project was supported by National Natural Science Foundation of China (NSFC Grant No. 32170431), the National Key R & D Program of China (Grant No. 2023YFC2605200) and the State Key Laboratory of Animal Biodiversity Conservation and Integrated Pest Management (SKLA2501).

Author contributions

BYZ: Conceptualization, Methodology, Formal analysis, Writing – original draft. DC: Writing – original draft, Writing – review and Editing, Supervision. RZY: Methodology, Data curation, Writing – review and Editing, Investigation. HJL: Writing – review and Editing, Data curation, Methodology. HLL: Writing – review and Editing, Data curation, Investigation. HX: Writing – review and Editing. CDZ: Writing – review and Editing, Supervision. ZYZ: Conceptualization, Writing – review and Editing, Funding acquisition.

Author ORCIDs

B. Zou https://orcid.org/0009-0000-5022-8669
D. Chesters https://orcid.org/0000-0001-7352-5770
R. Yu https://orcid.org/0009-0006-1318-260X
H. Liu https://orcid.org/0009-0000-3128-5684
H. Li https://orcid.org/0000-0003-2187-8536
H. Xiao https://orcid.org/0000-0002-8979-3793
C. Zhu https://orcid.org/0000-0002-9347-3178
Y. Zhang https://orcid.org/0000-0002-9713-5352

Data availability

The newly generated COI sequences have been deposited in GenBank under accession numbers PZ778155–PZ778363 and will be released publicly upon publication of this article or on 1 January 2027, whichever occurs first. Voucher specimens examined in this study are deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China. Collection, voucher, host-association, BOLD Process ID, and GenBank accession data are provided in Suppl. material 1.

Supplementary material 1

Specimen information of encyrtid parasitoids of psyllids. (> means male)

Authors: Boyu Zou, Douglas Chesters, Runze Yu, Huanji Liu, Hongliang Li, Hui Xiao, Chaodong Zhu, Yanzhou Zhang

Data type: xlsx

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Supplementary material 2

Detailed values of genus *Psyllaephagus*

Authors: Boyu Zou, Douglas Chesters, Runze Yu, Huanji Liu, Hongliang Li, Hui Xiao, Chaodong Zhu, Yanzhou Zhang

Data type: docx

Explanation note: **table S2-1.** Interspecific distance based on K2P model between 212 COI sequences. **table S2-2.** Intraspecific divergence based on K2P model between 212 COI sequences.

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Link: https://doi.org/10.3897/jhr.99.202665.suppl2

Supplementary material 3

Interspecific genetic distances

Authors: Boyu Zou, Douglas Chesters, Runze Yu, Huanji Liu, Hongliang Li, Hui Xiao, Chaodong Zhu, Yanzhou Zhang

Data type: docx

Explanation note: **table S3-1.** Interspecific distance based on K2P model between 263 28S-D2 sequences. **table S3-2.** Intraspecific divergence based on K2P model between 263 28S-D2 sequences.

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Link: https://doi.org/10.3897/jhr.99.202665.suppl3

Supplementary material 4

ASAP analysis

Authors: Boyu Zou, Douglas Chesters, Runze Yu, Huanji Liu, Hongliang Li, Hui Xiao, Chaodong Zhu, Yanzhou Zhang

Data type: docx

Explanation note: **table S4-1.** Settings and results of Automatic Barcode Gap Discovery (ABGD) analysis Kimura 2-parameter model (K2P),Jukes-Cantor substitution model (JC69), and Simple distance (p-distance) model. **table S4-2.** Results of Assemble Species by Automatic Partitioning (ASAP) analysis.

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Link: https://doi.org/10.3897/jhr.99.202665.suppl4

Supplementary material 5

The NJ trees of 113 COI haplotypes

Authors: Boyu Zou, Douglas Chesters, Runze Yu, Huanji Liu, Hongliang Li, Hui Xiao, Chaodong Zhu, Yanzhou Zhang

Data type: pdf

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Link: https://doi.org/10.3897/jhr.99.202665.suppl5

Supplementary material 6

The NJ tree of 81 28S-D2 rDNA haplotypes

Authors: Boyu Zou, Douglas Chesters, Runze Yu, Huanji Liu, Hongliang Li, Hui Xiao, Chaodong Zhu, Yanzhou Zhang

Data type: pdf

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