

# An Unidentified Parasitoid Community (Chalcidoidea) Is Associated With Pine-Feeding *Chionaspis* Scale Insects (Hemiptera: Diaspididae)

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**ABSTRACT** Many of the >22,000 described species of parasitoids in the Chalcidoidea attack armored scale insects, a group that may contain many cryptic species. Because the diversity of both these groups may be high, associations between them are likely to be underreported, and accurate reporting requires direct association between parasitoids and their scale insect hosts. One group of well-known armored scale insects are the pine-feeding *Chionaspis* of North America, which have been considered to be two species of economic pests, and have over a century of parasitoid records. These *Chionaspis* have been revealed to be a complex of at least 10, mostly cryptic, species. We have sequenced chalcidoid 28S rDNA from 50 parasitized *Chionaspis* hosts. We explore these host-parasitoid associations by placing the parasitoid sequences in a phylogenetic context. We aligned the sequences manually, based on secondary structure models, and added them to a previous 28S secondary structure alignment for the Chalcidoidea that includes 19 families, 72 subfamilies, 369 genera, and 685 species. The results of maximum likelihood analyses were interpreted using an updated, comprehensive list of parasitoids recorded from pine-feeding *Chionaspis*. The sequences are placed in nine clades, only two of which appear consistent with known *Chionaspis* parasitoids. Although pine-feeding *Chionaspis* are among the best-known armored scales, our results indicate that their parasitoid diversity has been underreported.

**KEY WORDS** Chalcidoidea, *Chionaspis pinifoliae*, *Encarsia*, parasitoid

Parasitoids in the superfamily Chalcidoidea number some 22,000–500,000 species (Heraty et al. 2012, Noyes 2013), and this diversity suggests there is much to be learned about parasitoid communities affiliated with a host. It is well known that chalcid parasitoids control populations of economically important species of armored scale insects (Hemiptera: Diaspididae) (Neumann et al. 2010), yet armored scales may include many cryptic species (Andersen et al. 2010, Gwiazdowski et al. 2011, Veà et al. 2012). Because of this cryptic diversity, host associations of many armored scale parasitoids may be erroneously recorded. One group of well-sampled armored scale insects with a documented community of parasitoids are the North American pine-feeding species in the genus *Chionaspis* Signoret (Burden and Hart 1993, Ben-Dov et al. 2013, Noyes 2013). These *Chionaspis* are economically important pests of pines (Miller and Davidson 2005) that outbreak (Luck and Dahlsten 1974, 1975), and correspondingly their parasitoid records span more than a century. Pine-feeding *Chionaspis* have long been thought to comprise two species, *Chionaspis heterophyllae* Cooley, and *Chionaspis pinifoliae* Fitch

(Miller and Davidson 2005, Watson 2005), but recent molecular work indicates that there are at least 10 species (Gwiazdowski et al. 2011), four of which have been recently described (Veà et al. 2012). The intensive collecting of *Chionaspis* described in Gwiazdowski et al. (2011) turned up many parasitized individuals and here we report an analysis of chalcidoid 28S sequences recovered from these. We place these 28S parasitoid sequences in the context of chalcidoid phylogeny (King et al. 2008) and compare their phylogenetic positions with the published record of parasitoids recovered from *C. heterophyllae* and *C. pinifoliae*. The unprecedentedly fine-grained phylogenetic analysis of the hosts (Gwiazdowski et al. 2011) provides a framework for exploring parasitoid–host associations and forming hypotheses about parasitoid diversity in this system.

## Materials and Methods

**Sample Collection and Sequence Preparation.** We collected *Chionaspis* from 51 species of pines across the United States and Mexico, and prepared genomic DNA from them using the Qiagen DNEasy Blood and Tissue Kit, as described in Gwiazdowski et al. (2011). When parasitoid pupae were found, DNA was prepared from individuals using the same methods. The primers used by Gwiazdowski et al. to amplify 28S rDNA sequences from *Chionaspis* (s3660, GAGAGTT-MAASAGTACGTGAAAC; and 28b, TCGGAAGGAAC-

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CAGCTACTA) also amplify hymenopteran 28S rDNA. The region amplified spans the D2–D3 expansion segments. We used these primers to amplify a  $\approx$ 850 bp fragment of 28S rDNA from 17 parasitoid pupae. In addition, we also incidentally obtained 33 sequences of chalcidoid 28S rDNA when attempting to amplify *Chionaspis* DNA, apparently owing to the undetected presence of chalcidoid larvae inside *Chionaspis* individuals, for a total of 50 chalcidoid 28S sequences. No rearing was attempted, and there are no adult parasitoid voucher specimens for these sequences. Voucher lots of cryopreserved scale insect hosts, which may contain parasitoids, are held at the Ambrose Monell Cryo Collection (AMCC) of the American Museum of Natural History. Collection information, including localities, and accession numbers for all *Chionaspis* parasitoid sequences and voucher lots is presented in Supp Table 1 [online only]. All *Chionaspis* parasitoid sequences tested negative for chimerism using the Bellepheron Web server (<http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl>; Huber et al. 2004). This test included three sequence pairs where repeated polymerase chain reaction (PCR) on scale insect genomic DNA yielded sequences from both the scale insect and its parasitoid. In GenBank, these pairs are—(1671A) Parasitoid: KF780921, Scale: GU349299.1; (D1522B) Parasitoid: KF780951, Scale: GU349160.1; (D1620B) Parasitoid: KF780922, Scale: GU349253.1.

**Secondary Structure-Based Sequence Alignment and Phylogenetic Analysis.** To provide a context for phylogenetic analysis of these unidentified parasitoid sequences, we used the manually aligned chalcidoid 28S sequence matrix (D2–D3 regions) of Munro et al. (2011), including sequences from Burks et al. (2011) and a single additional sequence from GenBank (AF254232.1, *Encarsia aurantii* (Howard 1894), D2 region only). The matrix includes sequences from identified specimens representing 19 chalcidoid families, 72 subfamilies, 369 genera, and 685 species (Burks et al. 2011, Munro et al. 2011), and spans the same region of 28S as our sequences. To assess whether additional relevant sequences were available, the top three BLAST-n (Altschul et al. 1990) sequence matches from GenBank for each *Chionaspis* parasitoid sequence were collected on 16 March 2013 using Geneious V6.1.2 (Geneious). All sequences had  $>99\%$  query coverage matching with  $\approx 90\%$  maximum identity to just 21 sequences, all of which are part of the Munro et al. (2011) alignment, including sequences used by Gillespie et al. (2005). These GenBank search results are presented in Supp Table 2 [online only].

The *Chionaspis* parasitoid 28S sequences were manually aligned using secondary structure models, according to the methods of Kjer (freely available from Karl Kjer's website at <http://rci.rutgers.edu/~insects/pdata.htm>) and Gillespie et al. (2005). These aligned sequences were then manually added to the alignment of Munro et al. (2011).

This combined alignment is equivalent to the “secondary structure-derived matrix with MAFFT-aligned RAA regions (SSME)” alignment by Munro et al. (2011) (used for their Fig. 1). Two different versions of this alignment were used for analysis; the first is the

alignment as described above (FULL alignment). The second replicated the Munro et al. (2011) conservative “core secondary structure-derived (SS) submatrix” (their SSNR alignment) by manually removing all regions of ambiguous alignment, including regions of expansion and contraction (REC), and regions of slip strand compensation, leaving only unambiguous, structurally aligned helices, core regions, and conserved blocks (SSONLY alignment).

Maximum likelihood bootstrap (ML) analyses were run on both alignments using the XSEDE resources, via the CIPRES computer portal (Miller et al. 2010). Following Munro et al. (2011), we used RAxML 7.3.2 with GTRGAMMA approximation of models, partitioned by region. Five thousand rapid bootstrap replicates were performed on each data set, and bootstrap percentages (BP) were drawn on the tree with the best-known likelihood score. Sequences from Munro et al. (2011) and Burks et al. (2011) retain their original names. Sequences recovered from scale insect specimens used by Gwiazdowski et al. (2011) follow their labeling where the letter S and number (e.g., S01 or S10) identifies the cryptic species of scale insect host for that sequence. Sequences not associated with Gwiazdowski et al. (2011) are labeled “undet”; all sequence names include an accession number with the *Pinus* species, and locality of the host scale insect.

**Taxonomic Estimation.** Table 1 presents a comprehensive synonymized list of parasitoids recorded from *C. heterophyllae* and *C. pinifoliae*. Current nomenclature is drawn from The Universal Chalcidoidea database (Noyes 2013). *C. pinifoliae* has been introduced to Europe, and those records are listed, but not considered here. The genera and species on this list are compared with the phylogenetic position of the specimens in this study to assess whether the phylogenetic positions are consistent with the taxonomic positions of known *Chionaspis* parasitoids. Tentative identifications are based on sister-group relationships and relative branch lengths. A singleton or clade that is nested within a monophyletic genus or higher taxon is hypothesized to be a member of that taxon. We consider relative branch lengths as a measure of heterospecific sequence divergence. Two individuals whose 28S sequences are identical are hypothesized to be conspecific. Branch lengths between individuals within a clade in the Munro et al. (2011) data set are interpreted as characteristic of heterospecific divergence within that clade. A singleton or clade on a similar-length or longer branch relative to identified species in a genus is hypothesized to belong to a species absent from the Munro et al. (2011) or Burks et al. (2011) data sets.

## Results

The topologies and branch lengths of the FULL and SSONLY alignments are consistent with each other and with the Chalcidoidea topology presented as the Fig. 1 of Munro et al. (2011). The FULL alignment, using the locally aligned regions of ambiguous alignment (RAAs) by Munro et al. (2011) has, overall, higher bootstrap support at all nodes than the SSONLY (RAA removed)

Table 1. A comprehensive list of parasitoids recorded from *C. heterophyllae* and *C. pinifoliae*

Parasitoid		References		Taxon included in phylogeny		Taxon matches results	
Family	Genus and Species	<i>C. het.</i>	<i>C. pinifoliae</i>	Genus	Species		
Aphelinidae	"Chalcid" undet						
	<i>Aphytis</i> sp. (Howard 1900)	21	2	NA	NA	Yes	Yes
	<i>Aphytis chilensis</i> ( <i>A. capitis</i> , <i>A. longiclavae</i> ) (Howard 1900)	25	15	Yes	NA	No	No
	<i>Aphytis diaspidis</i> (Howard 1881)		4 <sup>a</sup> , 11 <sup>a</sup> , 25, 26, 33	Yes	No	No	No
	<i>Aphytis (Aphelinus) mytilaspidis</i> (Le Baron 1870)	25	16, 18, 19, 25, 29	Yes	No	No	No
	<i>Aphytis proclia</i> (Walker 1839)	25, 28	1 <sup>a</sup> , 5, 16, 19, 24, 25, 28	Yes	No	No	No
	<i>Marietta mexicana</i> (Howard 1895)	21 <sup>b</sup> , 25, 34	25	Yes	No	No	No
	<i>Marietta pulchella</i> (Howard 1881) ( <i>Perissospterus pulchellus</i> )		8, 16, 25, 28	Yes	No	No	No
	<i>Coccobius (Phygadeuon) howardi</i> (Compere 1928)		22 <sup>a</sup> , 23 <sup>a</sup> , 25	Yes	No	No	No
	<i>Coccobius (Phygadeuon) varicornis</i> (Howard 1881)	25	11 <sup>a</sup> , 12 <sup>a</sup> , 16 <sup>a</sup> , 18, 19, 24 <sup>a</sup> , 25, 26, 28, 29, 31, 33	Yes	No	No	No
Coccophaginae	<i>Coccophagus flacifrons</i> (Howard 1885)		25, 31	Yes	No	No	No
	<i>Encarsia</i> sp. (Förster 1878)		28, 31	Yes	NA	Yes	Yes
	<i>Encarsia (Coccophagus) aurantii</i> (Howard 1894)		33 <sup>a</sup>	Yes	Yes	No	No
	<i>Encarsia (Prospaltella) bella</i> (Cahan 1927)	21 <sup>b</sup> , 34	6 <sup>a</sup> , 22 <sup>a</sup> , 23 <sup>a</sup> , 25, 31 <sup>a</sup>	Yes	No	Yes	Yes
	<i>Encarsia (Aspidiotiphagus) sp. near citrina</i> (Craw 1891)	25, 28 <sup>a</sup>	11 <sup>a</sup> , 28 <sup>a</sup>	Yes	Yes	Yes	Yes
	<i>Platygasterus</i> sp. (Crawford 1910) (potentially <i>Habrolepis</i> sp.)		28	Yes	No	No	No
	<i>Chrysoneuromyia (Acleris) schachtii</i> , <i>Closterocerus</i> <i>phenacapsia</i> (Yoshimoto 1972)		20 <sup>a</sup> , 22 <sup>a</sup> , 23 <sup>a</sup> , 25 <sup>a</sup> , 35 <sup>a</sup>	Yes	No	No	No
	<i>Dicoprus</i> sp. (Enock 1909)	28		No	NA	No	No
	The species below have been reported from outside North America, and are not considered here						
	<i>Ablertus (Azotus) atomon</i> (Walker 1847)		27 <sup>a</sup>	Yes	No	Austria	
Encyrtidae	<i>Ablertus (azotus) pinifoliae</i> (Mercet 1912)		11 <sup>a</sup> , 13	Yes	No	Spain	
	<i>Aphytis mytilaspidis</i> (Le Baron 1870)		3, 7	Yes	No	Germany, Spain	
	<i>Encarsia (Prospaltella) leucaspidis</i> (Mercet 1912)		11 <sup>a</sup> , 13	Yes	No	Spain	
	<i>Aphytis primus</i> (Mercet 1917) ( <i>Waterstonia prima</i> )		11 <sup>a</sup> , 14	Yes	No	Spain	
	<i>Metaphagus nadius</i> (Walker 1838) ( <i>Aphytis pinicola</i> )		11 <sup>a</sup> , 32	Yes	No	Spain	
	<i>Chlorocerus (Thysanus) conjugialis</i> (Mercet 1916)		11 <sup>a</sup> , 14	Yes	No	Spain	
	The references at right were not consulted, but are all reports outside of North America.						
			9, 10, 17, 30			Argentina, China, E-Europe, Germany	
Signiphoridae							

References are numbered by age, and full citations appear in the bibliography: 1 (Cooley 1899), 2 (Carnes 1906), 3 (Rühl 1913), 4 (Rust 1915), 5 (Britton 1922), 6 (Pettit 1925), 7 (Mercet 1930), 8 (Herrick 1931), 9 (De Santis 1940), 10 (Fulmek 1943), 11 (Thompson 1944), 12 (Cumming 1953), 13 (Thompson 1954), 14 (Kosztarab 1963), 15 (Martel and Sharma 1966), 16 (Martel and Sharma 1966), 17 (Nikolskaya and Yasnosh 1966), 18 (Sharma and Laviolette 1967), 19 (Martel and Sharma 1968), 20 (Yoshimoto 1972), 21 (Nielsen and Johnson 1973), 22 (Luck and Dahlsten 1974), 23 (Luck and Dahlsten 1975), 24 (Martel and Sharma 1975), 25 (Kronbein et al. 1979), 26 (Herting and Simmonds 1982), 27 (Darling and Johnson 1984), 28 (Shour 1986), 29 (Burden and Hart 1990), 30 (Huang and Polaszek 1998), 31 (Cooper and Cranshaw 1999), 32 (Guerrieri and Noyes 2000), 33 (Tooker and Hanks 2000), 34 (Fondren and McCullough 2005), 35 (Watson 2005).

<sup>a</sup> Junior synonyms appear in parentheses after the genus or species, respectively.

<sup>b</sup> Shour (1986) discovered specimen identifications published as *C. pinifoliae* later identified as *C. heterophyllae*.

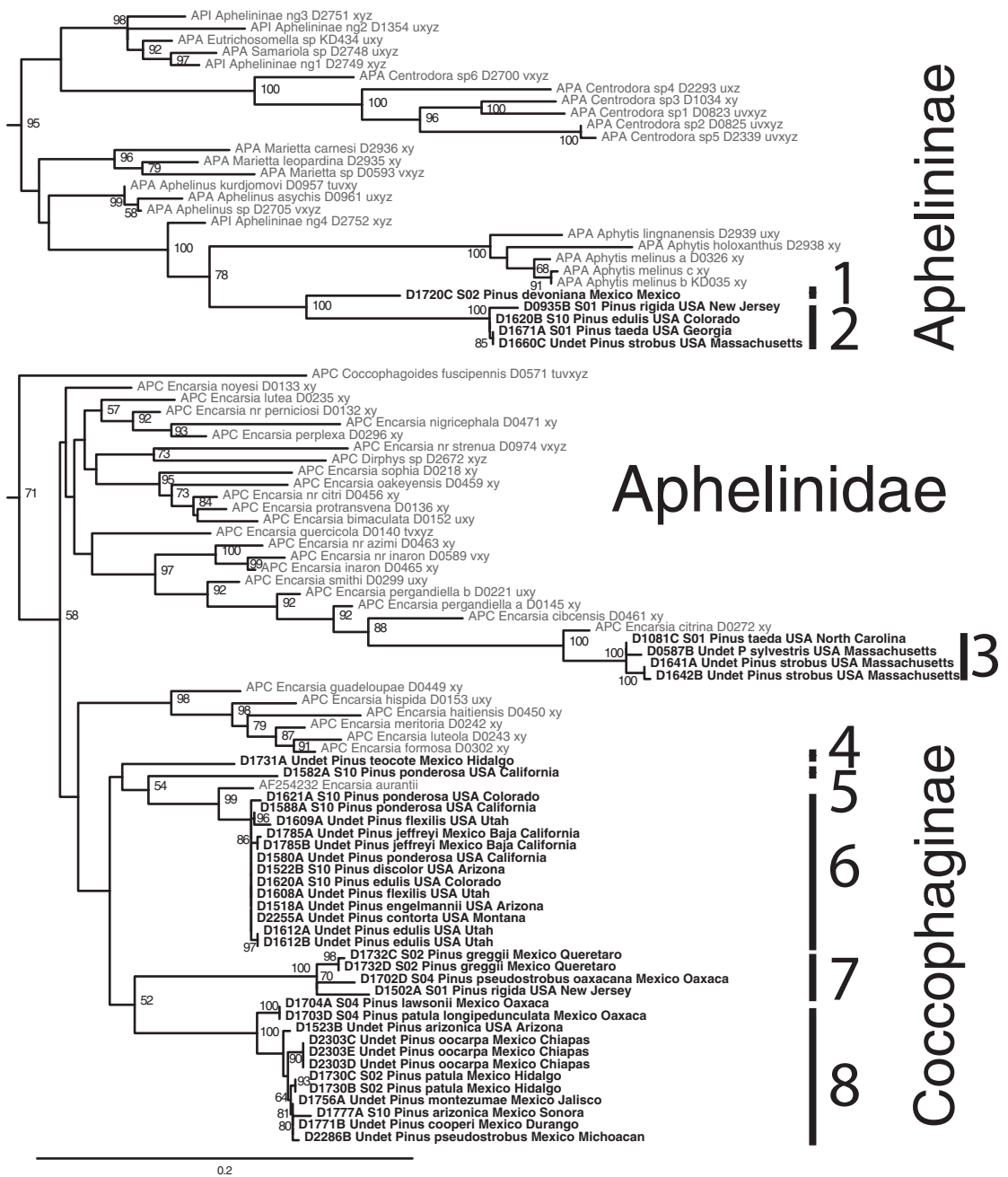


Fig. 1. Selected clades of aphelinid parasitoids, containing pine-feeding *Chionaspis* parasitoids, taken from a 28S (D2–D3 regions) secondary structure-based phylogeny of the Chalcidoidea including representatives from 19 families, 72 subfamilies, 369 genera, and 684 species, generated in an RAXML analysis with seed 38,652 and 5,000 rapid bootstrap replicates (support >50% shown). *Chionaspis* parasitoids appear in bold. Clades representing potential species are indicated by a vertical bar, and labeled with numbers 1 through 8. The scale bar represents estimated substitutions per site, taken from the entire chalcid topology.

alignment, and is used here for taxonomic estimation. Patterns of family or genera monophyly and bootstrap proportions across the SSONLY and FULL topologies are equivalent to those of Munro et al.'s (2011) SSNR and SSME alignments (reported in their Table 3); treefiles of both alignments are given in Supp Figs 1 and 2 [online

only]. Deep nodes have low BP, whereas nodes supporting families and genera relevant to specimens in this study are well supported (70–100%; Figs. 1 and 2); this pattern is consistent with using 28S to reconstruct a phylogeny of the Chalcidoidea (Gibson et al. 1999, Campbell et al. 2000, Gillespie et al. 2005, Munro et al.

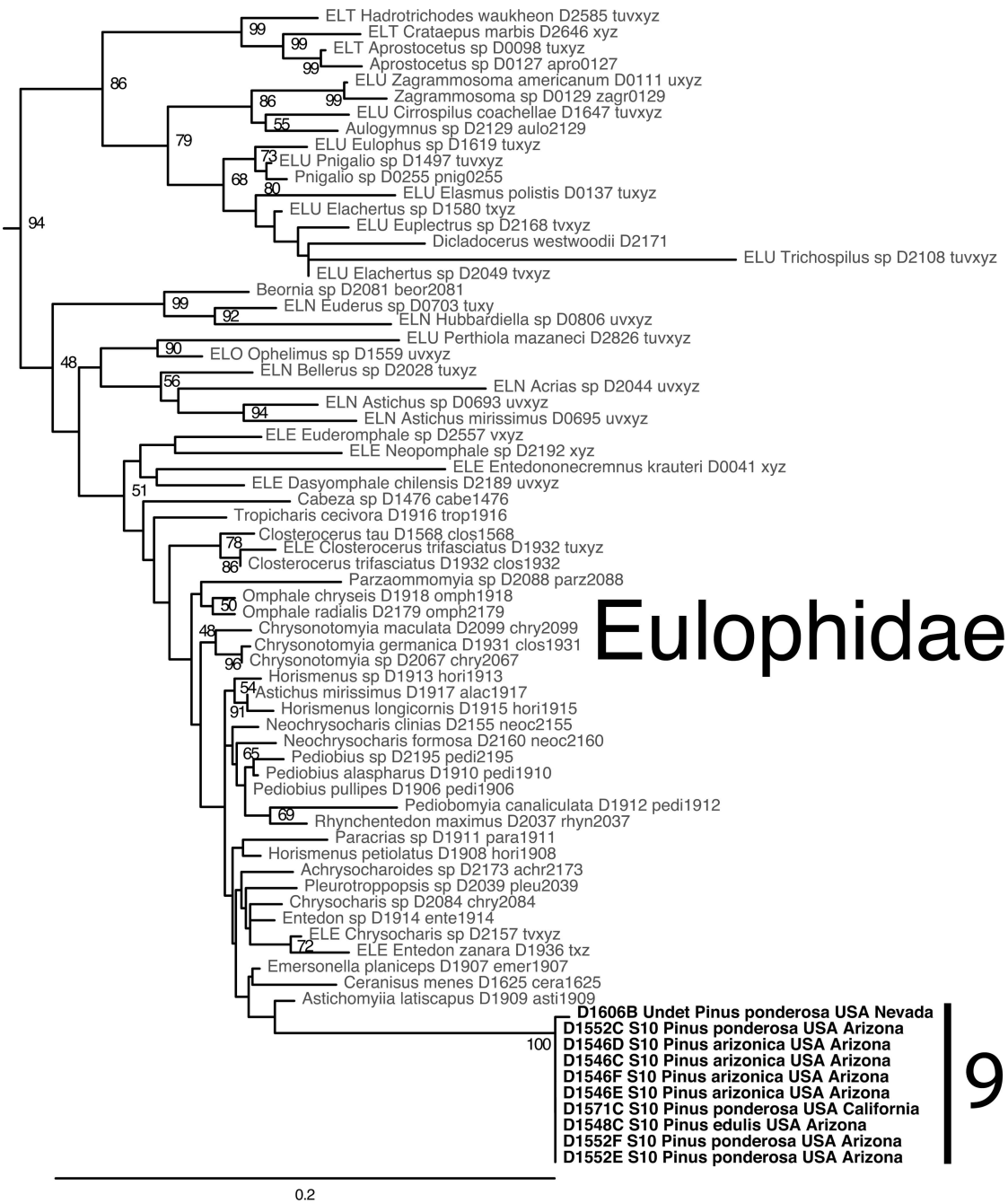


Fig. 2. Monophyletic clade of the Eulophidae, containing pine-feeding *Chionaspis* parasitoids as presented in Fig. 1. *Chionaspis* parasitoids appear in bold, and are indicated by a vertical bar labeled with the number 9. The scale bar represents estimated substitutions per site, taken from the entire chalcid topology.

2011). Sequences have been deposited in GenBank under numbers KF780915 through KF780964. The manual alignment of parasitoid sequences from this study, with base pair annotations per Gillespie et al. (2005) and Kjer (2004), along with an annotated FULL alignment, are given in Supp Tables 3 and 4 [online only]. The phylogeny includes seven of the eight genera listed in Table

1. The exception is *Dicopus* (Mymaridae), and none of the *Chionaspis* parasitoid sequences are nested within the strongly monophyletic (99% BP) Mymaridae. The phylogenetic analysis places the pine-feeding *Chionaspis* parasitoid sequences in nine clades, of which eight are within the Aphelinidae (Fig. 1), and one is within the Eulophidae (Fig. 2). In the Aphelinidae two



clades (Clades 1 and 2, Fig. 1.) are in the subfamily Aphelininae, and are not placed within any genera. In the subfamily Coccophaginae, six clades are placed in the genus *Encarsia* (Fig. 1.), and two of these clades can be tentatively associated with *Chionaspis* parasitoids using the literature from Table 1. The first (Clade 3), a sister group to *Encarsia citrina* (Craw), could be the "*Encarsia* near *citrina*" as reported by Krombein et al. (1979) and Shour (1986). The second (Clade 6) could be *Encarsia bella* (Gahan). Clade 6 is the sister group of *E. aurantii*; according to Gahan (1927) *E. bella* "also resembles *P. aurantii* Howard" (*Prospaltella* = *Encarsia*), and the specimen localities for Clade 6 are consistent with those for *E. bella* (central to western United States) as reported by Gahan (1927), Luck and Dahlsten (1974, 1975), and Cooper and Cranshaw (1999). The one eulophid clade (Clade 9, Fig. 2.) is not placed within any genus, and also occurs on a similarly long branch in the SSONLY topology.

### Discussion

The parasitoid community of pine-feeding *Chionaspis* has been considered as mostly a cosmopolitan group with a broad range of hosts (Burden and Hart 1993), yet only 2 of the 15 recorded species from North America (Table 1) appear to be consistent with the samples analyzed here. Certainly, the sampling for this study is biased by the stochastic nature of sequence collection, and possibly parasitoid biology. The sequence collection methods did not consider parasitoid life cycles, nor did they include adults; most sequences come from purified scale-insect genomic DNA, and this may preferentially bias recovery of endoparasitoids. *Chionaspis* parasitoids are hyper-, ecto-, and endoparasitoids, all of which are recovered at low density relative to their scale host (Nielsen 1970, Shour 1986). Endoparasitoids are recovered at densities of 15–30% of their scale host, whereas hyperparasitoids are only recovered at densities of <10% (Burden and Hart 1993). The genus *Aphytis* contains ectoparasitoids, and the singleton of Clade 1, relatively near *Aphytis* on the phylogeny, was recovered from a pupa. *Marietta mexicana* (Howard 1895) and *Marietta pulchella* (Howard 1881) are both hyperparasitoids, and their naturally low density may explain why they were not seen here.

The genus *Encarsia* is a large diverse group of endoparasitoids with many undescribed species (Heraty et al. 2008), and this is reflected in the diversity of the specimens sampled here. *Encarsia* in Clades 7 and 8 (Fig. 1) are mostly found from Mexico, an area known to be rich in *Encarsia* (Heraty et al. 2007), with ongoing descriptions of new species (Myartseva et al. 2012). Most known Mexican *Encarsia* are economically important (Myartseva et al. 2008, Lazaro-Castellanos et al. 2012), and while the specimens in Clades 7 and 8 (Fig. 1) could be one of these (without publicly available 28S sequence data), the authors are unaware of *Encarsia* from any armored scales on pines in Mexico, and these specimens could represent undescribed species.

Although most clades appear inconsistent with known *Chionaspis* parasitoids, we can use the conservative evolutionary rate of 28S (Hillis and Dixon 1991) to hypothesize about the biology for several of them. Intraspecific variation at 28S is expected to be low, and this pattern is consistent with the invariant sequences of Clade 9. At least 9 of the 10 Clade 9 sequences were recovered from the same *Chionaspis* species, S10 sensu Gwiazdowski et al. (2011), sampled from distant localities in the western United States (Arizona, California, and Nevada), suggesting that Clade 9 may represent a eulophid species that is specialized on *Chionaspis* species S10. Clade 7 shows an intriguingly large geographic and host range, and sequences D1703D and D1704A, which are clearly distinct within Clade 8, are recovered from Gwiazdowski et al.'s (2011) species S4, recently described as *Chionaspis caudata* (Vea et al. 2012; D1703D is the holotype of *C. caudata*). Furthermore, the Gwiazdowski et al. (2011) scale species S7 is a specialist on *Pinus strobus*, and the distinct differences of sequences D1641A and D1642B—from scales on *P. strobus*—hint at host specificity for these parasitoids (Feder and Forbes 2010). Future work on this and other such systems would benefit from the planned recovery of both host and parasitoid genetic markers from the same sample (Garipey et al. 2007, Traugott and Symondson 2008), preferably standardized ones (Rougerie et al. 2011). In this way, unidentified sources (parasitoid or scale) can be linked to identified ones, and compared using curated databases for armored scales (Ben-Dov et al. 2013), as well as Chalcidoid parasitoids (Noyes 2013), to clarify associations and highlight cryptic diversity.

Our results reveal new patterns of parasitoid diversity in the pine-feeding *Chionaspis* system, which is arguably one of the best studied groups of armored scales. These results suggest that parasitoid lists for even well-studied species of armored scale insects may be radically incomplete, and many scale–parasitoid associations await discovery.

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### References Cited

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215: 403–410.
- Andersen, J. C., B. B. Normark, G. E. Morse, and M. E. Gruwell. 2010. Cryptic diversity in the *Aspidiotus nerii* complex in Australia. *Ann. Entomol. Soc. Am.* 103: 844–854.
- Ben-Dov, Y., D. R. Miller, and G.A.P. Gibson. 2013. ScaleNet. (<http://www.sel.barc.usda.gov/SCALENET/classif.htm>).
- Britton, W. E. 1922. The pine leaf scale. *Chionaspis pinifoliae* Fitch. *Bull. Conn. Agric. Exp. Stn.* 234: 181–183.
- Burden, D. J., and E. R. Hart. 1990. Parasitoids of *Chionaspis pinifoliae* (Homoptera: Diaspididae) in Iowa. *Great Lakes Entomol.* 23: 93–97.
- Burden, D. J., and E. R. Hart. 1993. Parasitoids associated with *Chionaspis pinifoliae* and *Chionaspis heterophyllae* (Homoptera: Diaspididae) in North America. *J. Kans. Entomol. Soc.* 66: 383–391.
- Burks, R. A., J. M. Heraty, M. Gebiola, and C. Hansson. 2011. Combined molecular and morphological phylogeny of Eulophidae (Hymenoptera: Chalcidoidea), with focus on the subfamily Entedoninae. *Cladistics* 27: 581–605.
- Campbell, B., J. Heraty, K. Rasplus, K. Chan, J. Steffen-Campbell, and C. Babcock. 2000. Molecular systematics of the Chalcidoidea using 28S-D2 rDNA, pp. 59–73. *In* A. D. Austin and M. Dowton (eds.), *Hymenoptera: Evolution, Biodiversity and Biological Control*. CSIRO Publishing, Victoria, Australia.
- Carnes, E. K. 1906. The Coccidae of California; a descriptive list of the different scale insects found in and reported from California. W. W. Shannon, Sacramento, CA.
- Cooley, R. A. 1899. The coccid genera *Chionaspis* and *Hemichionaspis*, pp. 57. Hatch Experiment Station of the Massachusetts Agricultural College. Special bulletin. Carpenter & Morehouse, Amherst, MA.
- Cooper, D. D., and W. S. Cranshaw. 1999. The natural enemy complex associated with pine needle scale, *Chionaspis pinifoliae* (Fitch) (Homoptera: Diaspididae), in north central Colorado. *J. Kans. Entomol. Soc.* 72: 131–133.
- Cumming, M.E.P. 1953. Notes on the life history and seasonal development of the Pine needle scale, *Phenacaspis pinifoliae* (Fitch) (Diaspididae: Homoptera). *Can. Entomol.* 85: 347–352.
- Darling, D. C., and N. F. Johnson. 1984. Synopsis of Nearctic Azotinae (Hymenoptera: Aphelinidae). *Proc. Entomol. Soc. Wash.* 86: 555–562.
- De Santis, L. 1940. Sinopsis del genero *Physcus* Howard con descripción de una especie nueva (Hym., Chalcidoidea). *Revista de la Facultad de Agronomía de La Plata*. 24: 29–44.
- Feder, J. L., and A. A. Forbes. 2010. Sequential speciation and the diversity of parasitic insects. *Ecol. Entomol.* 35: 67–76.
- Fondren, K. M., and D. G. McCullough. 2005. Phenology, natural enemies, and efficacy of horticultural oil for control of *Chionaspis heterophyllae* (Homoptera: Diaspididae) on Christmas tree plantations. *J. Econ. Entomol.* 98: 1603–1613.
- Fulmek, L. 1943. Wirtsindex der aleyrodiden und cocciden parasiten. *Entomologische Beihefte* 10: 1–100.
- Gahan, A. B. 1927. Miscellaneous descriptions of new parasitic Hymenoptera with some synonymical notes. *Proc. United States Natl. Mus.* 71: 21–23.
- Gariepy, T. D., U. Kuhlmann, C. Gillott, and M. Erlandson. 2007. Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. *J. Appl. Entomol.* 131: 225–240.
- Geneious Computer Program. 2013. Geneious Computer Program, version R6 created by Biomatters. (<http://www.geneious.com>).
- Gibson, G.A.P., J. M. Heraty, and J. B. Woolley. 1999. Phylogenetics and classification of Chalcidoidea and Mymarommatodea: a review of current concepts (Hymenoptera, Apocrita). *Zool. Scr.* 28: 87–124.
- Gillespie, J. J., J. B. Munro, J. M. Heraty, M. J. Yoder, A. K. Owen, and A. E. Carmichael. 2005. A secondary structural model of the 28S rRNA expansion segments D2 and D3 for chalcidoid wasps (Hymenoptera: Chalcidoidea). *Mol. Biol. Evol.* 22: 1593–1608.
- Guerrieri, E., and J. S. Noyes. 2000. Revision of European species of genus *Metaphycus* Mercet (Hymenoptera: Chalcidoidea: Encyrtidae), parasitoids of scale insects. *Syst. Entomol.* 25: 147–222.
- Gwiazdowski, R. A., I. Veà, J. Andersen, C., and B. B. Normark. 2011. Discovery of cryptic species among North American pine-feeding chionaspis scale insects (Hemiptera: Diaspididae). *Biol. J. Linn. Soc.* 47: 47–62.
- Heraty, J., J. B. Woolley, and A. Polaszek. 2007. Catalogue of the *Encarsia* of the World. (<http://cache.ucr.edu/~heraty/Encarsia.cat.pdf>).
- Heraty, J. M., A. P. Polaszek, and M. Schauff. 2008. Systematics and Biology of *Encarsia*, pp. 71–87. *In* J. Gould, K. A. Hoelmer, and J. Goolsby (eds.), *Classical Biological Control of Bemisia tabaci in the United States: A Review of Interagency Research and Implementation*. Springer, The Hague, The Netherlands.
- Heraty, J. M., R. A. Burks, A. Cruaud, G.A.P. Gibson, J. Liljeblad, J. Munro, J.-Y. Rasplus, G. Delvare, P. Janšta, A. Gumovsky, et al. 2012. A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics* 29: 466–542.
- Herrick, G. W. 1931. Some shade-tree pests and their control. *Cornell Univ. Agric. Exp. Stn. Bull.* 515: 3–11.
- Herting, B., and F. J. Simmonds. 1982. A catalogue of parasites and predators of terrestrial arthropods. Section B. Enemy/host or prey, vol. II. Hymenoptera Terebrantia. Commonwealth Agricultural Bureaux., Slough, United Kingdom.
- Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Q. Rev. Biol.* 66: 411–453.
- Huang, J., and A. Polaszek. 1998. A revision of the Chinese species of *Encarsia* Förster (Hymenoptera: Aphelinidae): parasitoids of whiteflies, scale insects and aphids (Hemiptera: Aleyrodidae, Diaspididae, Aphidoidea). *J. Nat. Hist.* 32: 1825–1966.
- Huber, T., G. Faulkner, and P. Hugenholtz. 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* 20: 2317–2319.
- King, R. A., D. S. Read, M. Traugott, and W.O.C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. *Mol. Ecol.* 17: 947–963.
- Kjer, K. M. 2004. Aligned 18S and insect phylogeny. *Syst. Biol.* 53: 506–514.
- Kosztarab, M. 1963. The armored scale insects of Ohio (Homoptera: Coccoidea: Diaspididae), p. 120, vol. 2. *Bulletin of the Ohio Biological Survey*. Ohio State University, Columbus, OH.
- Krombein, K. V., J.P.D. Hurd, D. R. Smith, and B. D. Burks. 1979. *Catalog of Hymenoptera in America North of Mexico*. Smithsonian Institution, Washington, DC.

- Lazaro-Castellanos, C., H. Gonzalez-Hernandez, J. R. Lomeli-Flores, S. N. Myartseva, L. D. Ortega-Arenas, and S. Ocha-Ascencio. 2012. Natural enemies of armored scale (Hemiptera: Diaspididae) on avocado Hass in Michoacan, Mexico. *Rev. Colomb. Entomol.* 38: 6–13.
- Luck, R. F., and D. L. Dahlsten. 1974. Bionomics of pine needle scale, *Chionaspis pinifoliae*, and its natural enemies at south Lake Tahoe, California. *Ann. Entomol. Soc. Am.* 67: 309–316.
- Luck, R. F., and D. L. Dahlsten. 1975. Natural decline of a pine needle scale (*Chionaspis pinifoliae* Fitch) outbreak at south Lake Tahoe, California following cessation of adult mosquito control with malathion. *Ecology* 56: 893–904.
- Martel, P., and M. Sharma. 1966. Preliminary study of *Phenacaspis pinifoliae* in the eastern cantons of Quebec. *Annales de l'ACFAS*. 34: 66.
- Martel, P., and M. L. Sharma. 1968. Some details of the biology and ecology of the scale *P. pinifoliae* in Quebec. Quelques precisions sur la biologie et l'ecologie de la cochenille, *Phenacaspis pinifoliae* (Fitch), (Homoptera: Diaspididae), dans le Quebec. *Phytoprotection* 49: 19–25.
- Martel, P., and M. Sharma. 1975. Parasites de la cochenille du pin, *Phenacaspis pinifoliae* (Fitch), dans la région de Sherbrooke, Québec. *Annals of the Society of Entomology Québec* 20: 11–14.
- Mercet, R. G. 1930. Los aphelinidos de España. *Rev. de Biología Forestal y Limnología* (B) 2: 29–106.
- Miller, D. R., and J. A. Davidson. 2005. Armored scale insect pests of trees and shrubs (Hemiptera: Diaspididae). Cornell University Press, Ithaca, NY.
- Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, pp. 1–8, *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA.
- Munro, J. B., J. M. Heraty, R. A. Burks, D. Hawks, J. Mottern, A. Cruaud, J.-Y. Rasplus, and P. Jansta. 2011. A molecular phylogeny of the Chalcidoidea (Hymenoptera). *PLoS ONE* 6: e27023.
- Myartseva, S. N., E. Ruiz-Cancino, and J. M. Coronado-Blanco. 2008. *Encarsia aurantii* species-group (Hymenoptera: Aphelinidae), parasitoids of armored scales (Hemiptera: Diaspididae) in Mexico, with key and description of a new species. *Zoosystematica Rossica* 17: 67–71.
- Myartseva, S. N., E. Ruiz-Cancino, and J. Maria Coronado-Blanco. 2012. Two new species of *Encarsia foerster* (Hymenoptera: Aphelinidae) from the states of San Luis Potosi and Tabasco, Mexico. *Acta Zool. Mex. Nueva Ser.* 28: 457–464.
- Neumann, G., P. A. Follett, R. G. Hollingsworth, and J. H. de León. 2010. High host specificity in *Encarsia diaspidicola* (Hymenoptera: Aphelinidae), a biological control candidate against the white peach scale in Hawaii. *Biol. Control* 54: 107–113.
- Nielsen, D. G. 1970. Host impact, population dynamics, and chemical control of the pine needle scale, *Phenacaspis pinifoliae* (Fitch), in central New York. Cornell University, Ithaca, NY.
- Nielsen, D. G., and N. E. Johnson. 1973. Contribution to life-history and dynamics of Pine Needle Scale, *Phenacaspis pinifoliae*, Homoptera-Diaspididae in central New York. *Ann. Entomol. Soc. Am.* 66: 34–43.
- Nikolskaya, M. N., and V. A. Yasnosh. 1966. Aphelinidae of the European part of the USSR and Caucasus (Chalcidoidea, Aphelinidae) Akademii Nauk SSSR Zoologicheskogo Instituta Opredeliteli po Faune SSSR 91: 1–295.
- Noyes, J. S. 2013. Universal Chalcidoidea Database. World Wide Web electronic publication. (<http://www.nhm.ac.uk/chalcidoidea>) (accessed 10 January 2012–13 February 2013).
- Pettit, R. H. 1925. Report of the section of Entomology Experiment Station, p. 213. 63rd Annual Report of the State Board of Agriculture of Michigan 1923–24.
- Rougerie, R., M. A. Smith, J. Fernandez-Triana, C. Lopez-Vaamonde, S. Ratnasingham, and P.D.N. Hebert. 2011. Molecular analysis of parasitoid linkages (MAPL): gut contents of adult parasitoid wasps reveal larval host. *Mol. Ecol.* 20: 179–186.
- Rühl, M. 1913. Liste neuerdings beschriebener oder gezoogener Parasiten un ihrer Wirte. III (Fortsetzung.). *Societas Entomologica, Stuttgart* 28: 79–80.
- Rust, E. W. 1915. Three new species of *Aphelinus*. *Entomol. News* 26: 73–77.
- Sharma, M. L., and R. Laviolette. 1967. Present state of damage caused to pines by the coccid *P. pinifoliae* in Quebec/Etat actuel des dommages causes aux pins par la cochenille *Phenacaspis pinifoliae* Fitch dans le Quebec. *Phytoprotection* 48: 107–112.
- Shour, M. H. 1986. Life History studies of the Pine Scale *Chionaspis heterophyllae* Cooley and the Pine Needle Scale, *C. pinifoliae* (Fitch). Ph.D. dissertation. Purdue University, W. Lafayette, IN.
- Thompson, W. R. 1944. A catalogue of the parasites and predators of insect pests. Section 1, Parasite Host Catalogue, pp. 30. Part 3, Parasites of the Hemiptera. Commonwealth Agricultural Bureaux, Farnham Royal, United Kingdom.
- Thompson, W. R. 1953. Hosts of the Hymenoptera (Agonidae to Braconidae), pp. 10. A catalogue of the parasites and predators of insect pests. Commonwealth Institute of Biological Control, Ottawa, Ontario, Canada.
- Thompson, W. R. 1954. Hosts of the Hymenoptera (Calliceratid to Evaniid), pp. 271. A catalogue of the parasites and predators of insect pests. Commonwealth Agricultural Bureaux, Commonwealth Institute of Biological Control, Ottawa, Ontario, Canada.
- Tooker, J. F., and L. M. Hanks. 2000. Influence of plant community structure on natural enemies of pine needle scale (Homoptera: Diaspididae) in urban landscapes. *Environ. Entomol.* 29: 1305–1311.
- Traugott, M., and W.O.C. Symondson. 2008. Molecular analysis of predation on parasitized hosts. *Bull. Entomol. Res.* 98: 223–231.
- Veal, I., R. Gwiazdowski, and B. Normark. 2012. Corroborating molecular species discovery: four new pine-feeding species of *Chionaspis* (Hemiptera, Diaspididae). *ZooKeys* 270: 37–58.
- Watson, G. W. 2005. Diaspididae of the World, Arthropods of Economic Importance. ETI Bioinformatics: World Biodiversity Database, Unesco. University of Amsterdam, Holland, The Netherlands.
- Yoshimoto, C. M. 1972. New species of *Achrysocharis* (Eulophidae:Chalcidoidea) from pine needle scale (Diaspididae: Homoptera). *Can. Entomol.* 104: 1483–1485.

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