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Preliminary Phylogeny of *Encarsia* Förster (Hymenoptera: Aphelinidae) Based on Morphology and 28S rDNA

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Species of *Encarsia* Förster (Hymenoptera: Aphelinidae, Coccophaginae) are economically important for the biological control of whitefly and armored scale pests (Hemiptera: Aleyrodidae, Diaspididae). Whereas some regional keys for identification of *Encarsia* species are now available, few studies have addressed relationships within this diverse and cosmopolitan genus because of unreliable morphological data. Nuclear sequences of the D2 expansion region of 28S rDNA were determined from 67 strains of 24 species representing 10 species groups of *Encarsia*, 2 strains of *Encarsiella noyesi* Hayat, and 1 strain of *Coccophagoides fuscipennis* Girault. Analysis of molecular data alone and combined with morphological data resolves many nodes not resolved by morphology alone and offer insights into which morphological characters are useful for supporting group relationships. All analyses that include molecular data reveal *Encarsia* to be paraphyletic with respect to *Encarsiella*. If monophyly of *Encarsia* is constrained, the relationships are the same but with a different root within *Encarsia*, and these trees are presented as an alternate hypothesis. The *luteola* and *strenua* species groups are shown by both morphological and molecular data to be monophyletic, whereas the *inaron* group, the *E. nigricephala* + *luteola* group, and the *E. quericola* + *strenua* group are supported only by molecular data. The *aurantii* and *parvella* species groups are not supported in any of the analyses. The utility of morphological characters for defining species group relationships is discussed. © 2001 Academic Press

***Key Words:* *Encarsia* phylogeny; 28S ribosomal DNA; morphology; Aphelinidae; biocontrol.**

INTRODUCTION

The genus *Encarsia* Förster (Hymenoptera: Aphelinidae, Coccophaginae) is a diverse and cosmopolitan group of species with great economic importance. Adults are minute wasps (1–2 mm) that parasitize various sessile Hemiptera or other Aphelinidae (Viggiani, 1984). At present, 267 valid species of *Encarsia* are recognized (J. B. Woolley and J. M. Heraty, unpublished data), although many species remain undescribed. Species of *Encarsia* are extensively collected and utilized for control of whiteflies and armored scales (Hemiptera: Aleyrodidae, Diaspididae), which are among the most destructive crop pests (Kajita *et al.*, 1992; McAuslane *et al.*, 1993; Chou and Lin, 1993; Heinz and Parella, 1994; Tzeng and Kao, 1995; Liu and Stansly, 1996; Goolsby *et al.*, 1998; Toscano *et al.*, 1998). Whereas new species are continually being described and categorized as part of these biological control programs (Evans, 1997; Evans and Castillo, 1998; Evans and Polaszek, 1997, 1998), relatively little attention has been paid to the relationships between species within *Encarsia* and the delimitation of monophyletic groups.

A common assumption in choosing species as biological control agents is that closely related species share the same habits and host preferences, and phylogenetic relationships might be used to assess desirable candidates. For example, within all of *Encarsia*, only the 4 species included in a monophyletic *flavoscutellum* group are known to attack Hormaphidiidae (Aphidoidea) (Evans *et al.*, 1995). Such information can be used effectively to narrow the search for new control agents of a pest group. However, misunderstanding species relationships can lead to misconceptions about behavior and host associations. A case in point is the *aurantii* species group, which currently includes 40 described species of *Encarsia* that are morphologically similar, although quite diverse in their behavior. For example, *E. smithi* Silvestri is a parasite of whiteflies, other species of *Encarsia*, and itself (as a heteronomous hyperparasitoid); *E. aurantii* Howard is a parasite of armored scales; and *E. perniciosi* Tower parasitizes armored scales and itself (Table 1).

Before the 1970's, most species now assigned to *Encarsia* were generally allocated to *Encarsia* Förster 1878, *Prospaltella* Ashmead 1904, and *Aspidotiphagus* Howard 1894. *Encarsia* were considered to be whitefly parasites, and *Aspidiotiphagus* and *Prospaltella* were considered to be armored scale parasites. All of these

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genera were synonomized under *Encarsia* by Viggiani and Mazzone (1979). The synonymy of *Aspidiotiphagus*, a morphologically discrete group of armored scale parasites, was disputed and a new genus erected, *Aleurodiphilus* DeBach and Rose 1981, for similar species attacking whiteflies which shared a narrow fore wing with a distinct asetose patch (DeBach and Rose, 1981). Hayat (1983) treated all four genera as *Encarsia*, which has been the recognized convention to date.

To accommodate such a large and heterogenous assemblage of species, there has been an attempt to create species groups as representations of morphologically similar taxa. Ideally, placement of species into species groups would be determined primarily by shared derived morphological characters. Unfortunately, species groups of *Encarsia* are often defined by combinations of characters of unknown polarity, many of which are also characteristic of one or more species found in other species groups (Viggiani and Mazzone, 1979). For example, the close placement of scutellar sensilla, a character once considered diagnostic of the *strenua* species group, is now known to be convergent and is found in at least three unrelated groups of species (J. M. Heraty and A. Polaszek, unpublished). Thirty-one species group names have been proposed, although only 16 species groups currently are recognized (Hayat, 1998). As illustrated by several examples in Table 1, the placement of species of *Encarsia* into species groups has a muddled history with extensive and frequent changes taking place throughout the past two decades. In part the problem is the characterization of groups based on plesiomorphic or homoplasious character states.

Here we review morphological characters useful in evaluating *Encarsia* species and present a morphological analysis of 24 species of *Encarsia*. The analysis of nucleotide sequence data provides an opportunity to test the validity of morphological features currently used in placing species of *Encarsia* into species groups. Sequences of the D2 expansion region of 28S rDNA are used to evaluate the phylogenetic results of the molecular data separately and in combination with morphological data. While not intended as a comprehensive survey of the genus *Encarsia*, the aim of this study is to offer new insights into the utility of morphological characters for classifying species of *Encarsia* and lay the foundation for resolution of phylogenetic relationships within the genus.

MATERIALS AND METHODS

Specimens

Sixty-seven strains of 24 species of *Encarsia* allocated to 10 species groups (*E. quercicola* (Howard) not placed) were included in this analysis (Table 1). Identifier codes were assigned to different collections to distinguish multiple strains of the same species. A list of *Encarsia* specimens, their code, their location of origin, and their contributors is provided in Appendix 1. Species listed as "nr" (near) could not be accurately placed within the associated species based on morphological traits. *Encarsia* n. sp. is an undescribed species from Queensland, Australia. For outgroup comparisons, two strains of *Encarsiella noyesi* Hayat, D133 and D141, were supplied by T. Bellows of the University of California, Riverside; and the sequence of *Coccophagoides fuscipennis* Girault was provided by Bruce Campbell, USDA. *Encarsia*, *Encarsiella*, and *Coccophagoides* are all members of the Pteroptricini (Aphelinidae: Coccophaginae: Pteroptricini), with *Encarsiella* and *Dirphys* considered potential sister groups of *Encarsia* (Polaszek and Hayat, 1992). All taxa used in this study are represented by voucher specimens from the same culture or strain that are deposited at the University of California, Riverside (UCR) under voucher code HR3 or at the Australian National Insect Collection, Canberra, Australia (ANIC).

Character Analysis

Multiple representatives of each species, as available, were digested overnight in 10% KOH and slide-mounted in Canada Balsam. Specimens were examined with a Leica DMRB compound microscope with differential interference contrast optics. Photographic images are compilations of several pictures captured at different focal depths and merged into a single in-focus image using the Automontage System (Synoptics Ltd.).

Morphological characters were scored for all species using the representative material on hand. It is difficult to identify and score morphological features for the various species or groups within *Encarsia* due to the problem of scoring exemplar species that may not represent the full range of variation within a group. Another problem of choosing exemplar species is that some group characters, such as the wrinkling of the basal gastral tergite in the *citrina* group, would be an autapomorphy of the single representative in our analysis, *E. citrina* (Craw), although across *Encarsia* it is both synapomorphic for the *citrina* group and, at the same time, homoplasious with species in other groups, such as *E. diaspidicola* (Silvestri) in the *strenua* group (cf. discussion in DeBach and Rose, 1981). Additionally, we avoided characters that we know vary within larger species groups (i.e., *strenua* group), such as relative tarsus length, body pigmentation, and relative ovipositor length. With this in mind, we were able to score 14 characters (Appendix 2) that define *Encarsia* and some of the relationships within the group. Most character state definitions follow Polaszek and Hayat (1992), although a few others that better define species groups within *Encarsia* and that define more than one taxon within the analysis have been extracted from the

TABLE 1

Host Associations and Species Group Placement for Species of *Encarsia* Used in This Study

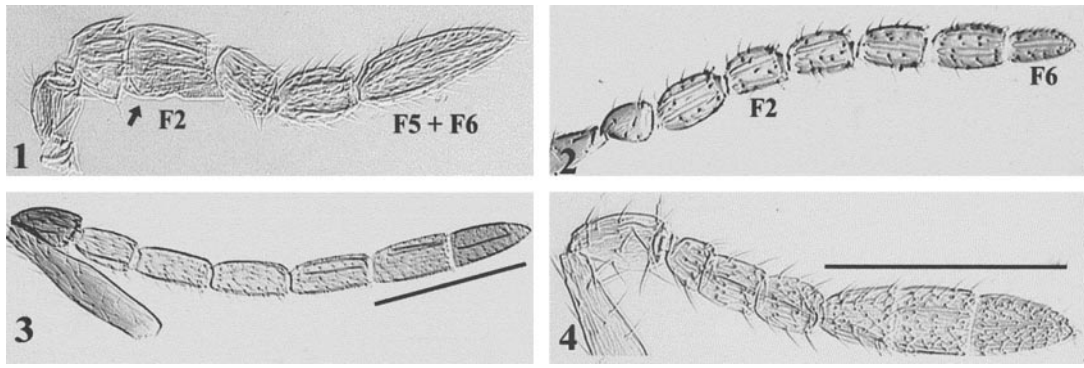
Species group Species	Host association	Former species group placement where applicable	References
aurantii group			
E. aurantii (Howard)	Diaspididae[a]	*aurantii*	Viggiani and Mazzone, 1979; Hayat, 1989, 1998.
E. perniciosi (Tower)	Diaspididae Aphelinidae	*aurantii*	Hayat, 1989, 1998; Huang and Polaszek, 1998.
E. smithi (Silvestri)	Aleyrodidae	*aurantii*	Hayat, 1998.
	Aphelinidae	Undetermined	Huang and Polaszek, 1998.
		opulenta or *aurantii*	Hayat, 1989.
		smithi	Viggiani and Mazzone, 1979.
citrina group (= *Aspidiotiphagus*)			
E. citrina (Craw)	Diaspididae	*citrina*	Viggiani and Mazzone, 1979; Hayat, 1989, 1998.
		=*Aspidiotiphagus*	DeBach and Rose, 1981.
cubensis group			
E. nigricephala Dozier	Aleyrodidae	*cubensis*	Polaszek *et al.*, 1992.
		?*duoronga*	Hayat, 1998.
inaron group			
E. nr *azimi* Hayat	Aleyrodidae	*inaron*	Hayat, 1989, 1998; Huang and Polaszek, 1998.
		coryli	Viggiani, 1981; Lopez-Avila, 1987.
E. inaron (Walker)	Aleyrodidae Aphelinidae	*inaron*	Hayat, 1989, 1998; Huang and Polaszek, 1998.
lahorensis/perflava group			
E. cibcensis Lopez-Avila	Aleyrodidae	*lahorensis*	Hayat, 1998.
		perflava	Hayat, 1989; Huang and Polaszek, 1998.
		lutea	Lopez-Avila, 1987.
lutea group			
E. lutea (Masi)	Aleyrodidae	*lutea*	Viggiani and Mazzone, 1979; Hayat, 1989, 1998; Huang and Polaszek, 1998.
luteola group			
E. formosa Gahan	Aleyrodidae	*luteola*	Gahan, 1924; Polaszek *et al.*, 1992.
		?*inaron*	Hayat, 1998.
		formosa	Viggiani and Mazzone, 1979; Hayat, 1989.
E. guadeloupae Viggiani	Aleyrodidae Diaspididae	*luteola*	Polaszek *et al.*, 1992.
E. haitiensis Dozier	Aleyrodidae	*luteola*	Polaszek *et al.*, 1992.
E. hispida De Santis	Aleyrodidae	*luteola*	Polaszek *et al.*, 1992.
E. luteola Howard	Aleyrodidae	*luteola*	Polaszek *et al.*, 1992.
E. meritoria Gahan	Aleyrodidae	*formosa*	Viggiani and Mazzone, 1979.
		luteola	Polaszek *et al.*, 1992.
opulenta group			
E. perplexa Huang & Polaszek	Aleyrodidae	*opulenta*	Huang and Polaszek, 1998.
parvella group			
E. mineoi Viggiani	Aleyrodidae	*parvella*	Hayat, 1989; Polaszek *et al.*, 1992.
E. pergandiella Howard	Aleyrodidae	*parvella*	Hayat, 1989, 1998; Polaszek *et al.*, 1992.
		pergandiella	Viggiani and Mazzone, 1979; Viggiani, 1993.
		=*Aleurodiphilus*	DeBach and Rose, 1981.
strenua group			
E. bimaculata Heraty & Polaszek	Aleyrodidae	*strenua*	Heraty and Polaszek, 2000.
E. nr *citri* Ishii	Aleyrodidae		
E. n. sp.	Aleyrodidae[b]		
E. protransvena Viggiani	Aleyrodidae	*strenua*	Hayat, 1989, 1998; Huang and Polaszek, 1998.
E. sophia (Girault)	Aleyrodidae[c]	*strenua*	Polaszek *et al.*, 1992; Hayat, 1998; Huang and Polaszek, 1998.
		lahorensis	Hayat, 1989.
Undetermined group			
E. quercicola (Howard)		Undetermined	J. M. Heraty and A. Polaszek, unpublished.
		strenua	Evans, 1993.
		aurantii	Hayat, 1989, 1998.

Note. Host associations from Noyes (1998).

[a] Reported as a parasite of *Aleuroplatus* (=*Aleyrodes*) by Howard (1908); probably erroneous.

[b] S. Schmidt, CSIRO, personal communication.

[c] A record for Diaspididae was regarded as erroneous, although other host records from Psyllidae and Aphididae could be a result of hyperparasitic males (Heraty and Polaszek, 2000).



FIGS. 1–4. Antenna of *Encarsia:* (1) *E. nigricephala,* male; (2) *E. bimaculata,* male; (3) *E. meritoria,* female; (4) *E. lutea,* female. F, flagellomere; bar indicates segments of clava.

literature. Morphological terms follow Hayat (1998), except that metasomal tergites are counted such that the seventh metasomal tergite is the spiracle-bearing tergite.

Character 1. Number of antennal flagellomeres (F) in males: 5, F5 and F6 fused (Fig. 1); 6, all segments unfused (plesiomorphic) (Fig. 2). The males of three species, *E. citrina, E. guadaloupae* Viggiani, and *E. haitensis* Dozier, are unknown and were scored as missing. Within the *citrina* group, a male is known only for *E. agilior* De Santis, which has six flagellomeres (state 0), but because of considerable within-group variation we did not extrapolate scores between species within a group.

Character 2. Sensorial complex on basal antennal flagellomeres of males: 0, absent (Fig. 2); 1, present (Fig. 1, arrow). In most males of *Encarsia,* the basal flagellomeres are undifferentiated (state 0). However, in males of the *cubensis, lutea,* and *opulenta* groups, the basal two to three flagellomeres are swollen, and the ventral margin of the second flagellomere is impressed and associated with a distinct glandular complex (state 1) (Silvestri, 1928; Viggiani and Mazzone, 1982; Viggiani and Laudonia, 1989; Polaszek *et al.*, 1992; Viggiani, 1996; Evans and Polaszek, 1998). The form of the complex varies distinctly between species groups, but for an initial and likely over-simplified hypothesis we coded this as one feature. This "simple" coding might tend to support an artificial assemblage of species, although it turns out not to be the case, as discussed later. In *E. perniciosi,* a patch of specialized sensilla occurs on the ventral surface of F2 (Viggiani and Laudonia, 1989), but it is not associated with a specialized structure and hence not treated as a derived state, although the initial hypothesis of only two character states is later modified.

Character 3. Shape of the female basal flagellomere: 0, elongate, longer than broad (Fig. 3); 1, compressed, at most as long as broad, usually shorter (Fig. 4). This character is usually straightforward with the basal segment transverse or broader than long (state 1).

Character 4. Number of female claval segments: 2 (Fig. 3); 3, plesiomorphic (Fig. 4). The apical two or three segments of the antennal flagellum may form a clava in which the segments are partially or completely fused for most of their width. Segments not fused are joined only by a narrow peduncle and in slide preparations are clearly separated for most of their width. The fusion is often associated with a swelling of the apical segments into a distinct clava or club. However, the clava may be undifferentiated with no swelling and all segments appearing segregated. In these cases, the apical segments in slide mounts are broadly joined and three-segmented in all of the taxa scored for this analysis.

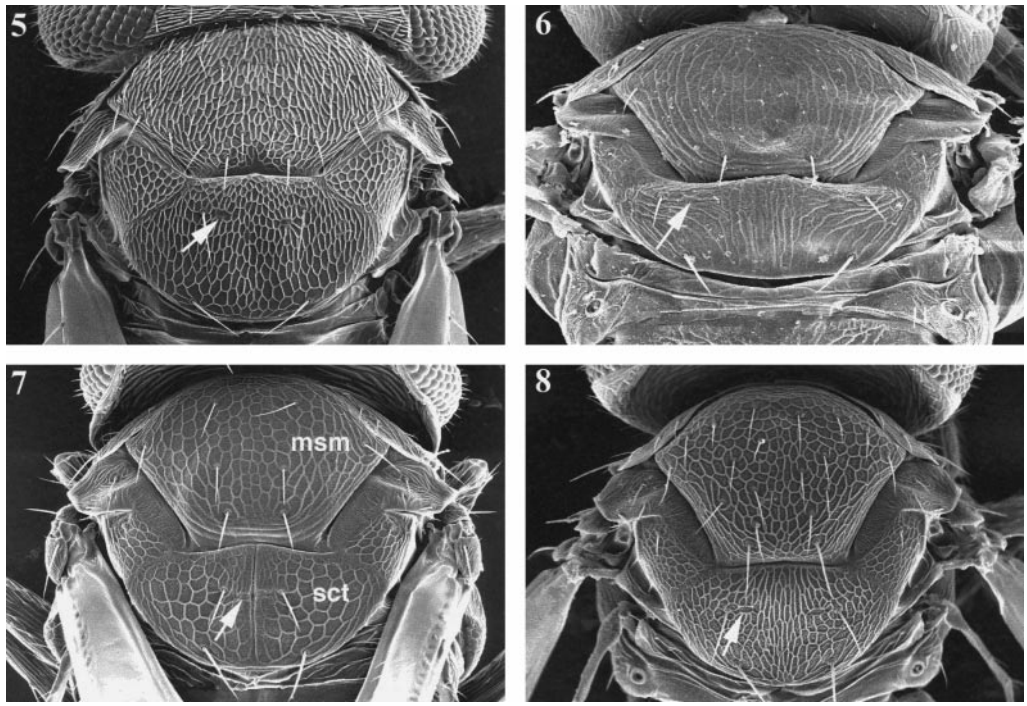
Character 5. Maxillary palpi: 1 or 2 palpomeres. A two-segmented palpus (plesiomorphic) has been observed (but not verified by us) only in *E. pulliclava* and *E. tricolor* Förster (Polaszek and Hayat, 1992). All *Encarsia* examined for this study had only a single palpomere.

Character 6. Number of setae on midlobe of mesoscutum (Polaszek and Hayat, 1992): 0, more than 20 (Fig. 5); 1, 6–18 (Figs. 7 and 8); 2, fewer than 6 (Fig. 6).

Character 7. Scutellar sculpture (Polaszek and Hayat, 1992): 0, cellulate sculpture uniform in size across scutellum (Fig. 5); 1, cells compressed and elongate medially (Figs. 6–8).

Character 8. Position of scutellar sensilla: 0, far apart (Figs. 5, 6, and 8); 1, separated by less than twice the diameter of the sensillum (Fig. 7). Different conditions occur for this state and the homology of each condition remains questionable.

Character 9. Number of tarsomeres: 4 (Fig. 9); 5, plesiomorphic (Fig. 10). Reduction from a five- to a



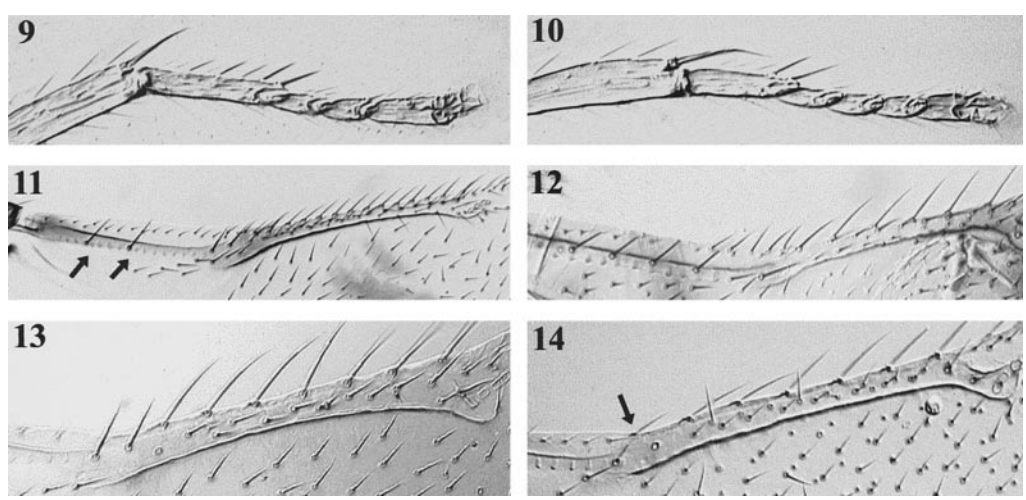
FIGS. 5–8. Scutellum and mesoscutum: (5) *Encarsiella noyesii;* (6) *Encarsia citrina;* (7) *Encarsia sophia;* (8) *Encarsia formosa.* msm, mesoscutal midlobe; sct, scutellum; arrow points to scutellar sensilla.

four-segmented tarsus is a derived trait, but since it represents a simple reduction, the value of this as a character for delimiting natural groups was questioned by Hayat (1998).

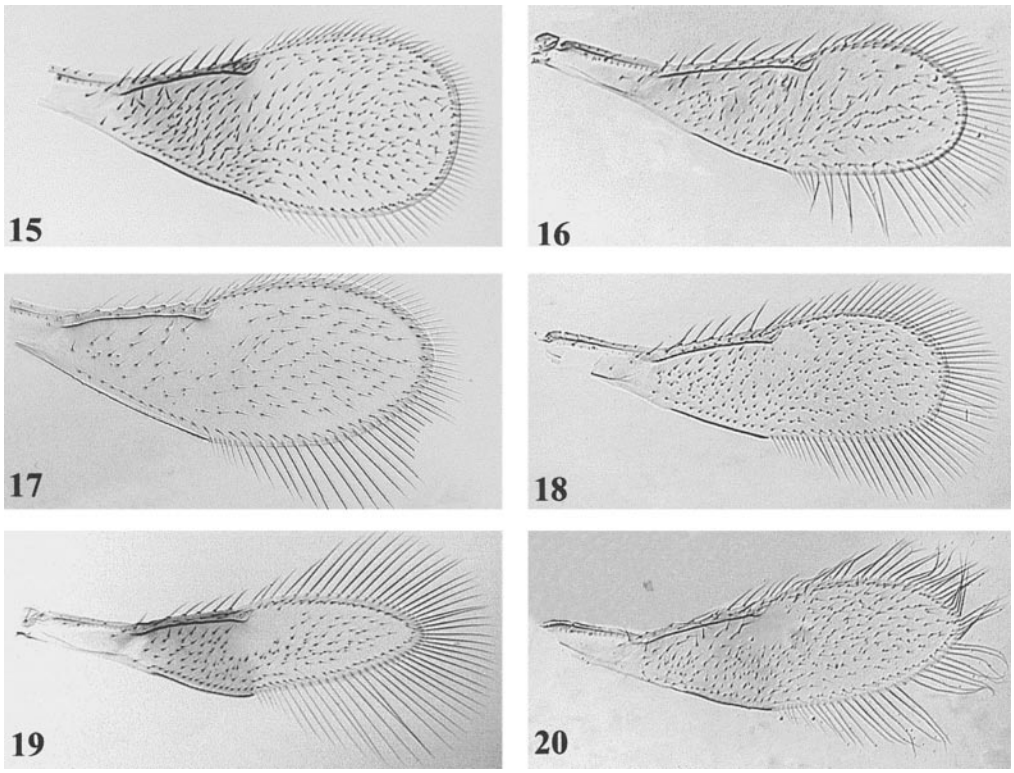
Character 10. Number of setae on submarginal vein (Polaszek and Hayat, 1992): 2 (Fig. 11); 3 or more (Fig. 12). Polarity is uncertain for this character.

Character 11. Marginal costal cell setae (Heraty and Polaszek, 2000): 0, no specialized setae present (Figs. 11–13); 1, 1–3 setae located apically and emerging from the wing margin (Fig. 14, arrow), not the wing surface.

Character 12. Asetose patch on fore wing: 0, wing uniformly pilose (Fig. 15); 1, wing with asetose patch (Figs. 16–20). Typically, the *citrina*, *parvella*, and *cubensis* groups have the setae clearly absent in an area surrounding and posterior to the stigmal vein. In *E. cibcensis* Lopez-Avila, the bare patch is spread



FIGS. 9–14. (9 and 10) Mid-tarsus of *Encarsia:* (9) *E. formosa;* (10) *E. citrina.* (11–14) Submarginal vein: (11) *Encarsiella noyesii;* (12) *Coccophagoides fuscipennis;* (13) *Encarsia* nr *azimi;* (14) *Encarsia* nr *citri.*



FIGS. 15–20. Fore wing of *Encarsia:* (15) *E. smithi;* (16) *E. cibcensis;* (17) *E. nigricephala;* (18) *E. mineoi;* (19) *E. citrina;* (20) *E. pergandiella.*

around the anterior margin of the wing beyond the stigmal vein and along the posterior margin. For simplicity, both conditions were coded as the same derived state, and in this case, the hypothesis of homology in *E. cibcensis* and the *citrina* and *parvella* groups is later supported.

Character 13. Width of fore wing: 0, broadly rounded (Figs. 15–18); 1, narrow and elongate, anterior margin sometimes curved (Figs. 19 and 20).

Character 14. Number of setae on metasomal tergite 7: 2 setae; 4 or more setae medial to cerci (plesiomorphic). *Coccophagoides* and most *Encarsia* have six setae on metasomal tergite 7: two minute setae lateral to the cerci and adjacent to the spiracles and four prominent setae medial to the cerci. The minute setae are absent only in *E. mineoi* Viggiani. *Encarsiella* have a variable number of medial setae but always more than four (Polaszek and Hayat, 1992). Two medial setae occur in several species, including *E. sophia* (Girault), in which the character state is considered unique within the *strenua* group (Heraty and Polaszek, 2000).

DNA Extraction and Sequencing

Adults were either fresh-frozen and stored at −80°C or killed directly in 95% EtOH. The majority of DNA extraction, PCR, and sequencing was as described in Babcock and Heraty (2000). Additionally, several species were sequenced directly from PCR product at the San Diego State University Microchemical Core Facility, and several species were sequenced independently in the De Barro laboratory (Appendix 1). Sequences of each strand were aligned using the ClustalW algorithm in MacVector and any ambiguities resolved by eye. Alignments were made on the complete data set (all populations) and not changed after removal of redundant populations for reduced analyses. One or two clones were sequenced from each individual and one to three individuals sequenced from each population. Sequences have been deposited in the GenBank database under Accession Nos. AF223366–AF223378 (Babcock and Heraty, 2000) and AF254192–AF254248.

Phylogenetic Analysis

Parsimony analyses were performed on the morphological data individually and in combination with the nucleotide data (see below). PAUP 4.0b3a (Swofford, 2000) was used for a heuristic search using random stepwise addition and 50 replicates. All characters were treated as unweighted and unordered. Tree–Bisection–Reconnection (TBR) was used and a single tree held at each step during stepwise addition. Numerous trees resulted from analysis of

the morphological data. To choose a more resolved set of trees from this set, and to test the stability of these analyses, we reanalyzed the data using successive approximations character weighting (SAW) (Farris, 1969; Carpenter, 1988). Characters were reweighted using the maximum value of the rescaled consistency index and a base weight of 1000. After SAW, characters were reweighted to one and the tree length was compared to that of the original unweighted tree, an identical value meaning that SAW selected one or more of the most-parsimonious trees. Bootstrap support for nodes was estimated using 100 replicates (Felsenstein, 1985, 1988). Bremer support values were calculated using TreeRot Version 2.0 (Sorenson, 1999) and manually for constraint analyses.

Genetic distances within and between species were calculated as the average number of differences divided by the number of shared nucleotides for all possible pairwise comparisons. Because of the near identity of multiple representatives of each species, a reduced data set (26 taxa) that included only one sequence per species was used. The reduced data set was produced for two reasons: to observe the effect on relationships of multiple terminal taxon representatives versus single exemplars (populations) and to simplify the combined molecular and morphological analysis. Strains were chosen at random and are noted by asterisks in Appendix 1.

Concordance of different phylogenetic reconstruction methods such as maximum-parsimony (MP) and maximum-likelihood (ML) is seen as a way to test the accuracy of phylogenetic estimation, especially when branches are long enough to attract and where methods might be differentially sensitive to long branches (Kim, 1993; Huelsenbeck, 1997). Maximum-likelihood searches were performed on the complete and the reduced molecular data sets using PAUP4.0b3a. Likelihood ratio tests were used to suggest a model with the best likelihood score (Modeltest 3.0; Posada and Crandall, 1998). For both the full and the reduced data sets, the suggested model was general reversible substitution, with estimated base frequencies and among-site rate variation (TrN+I+Γ) (Tamura and Nei, 1993). Likelihood parameters for heuristic searches were estimated using Modeltest 3.0 (Posada and Crandall, 1998).

The partition homogeneity test (PAUP 4.0b3a) was used to test for incongruence between the morphological and the molecular data sets. One hundred replicates were performed with a resulting P value of <0.1 considered significant. Trees resulting from the unconstrained and constrained analyses of the combined data were compared using the Kishino–Hasagawa test, the Templeton test, and the Compare2 T-PTP test as implemented in PAUP4.0b3a.

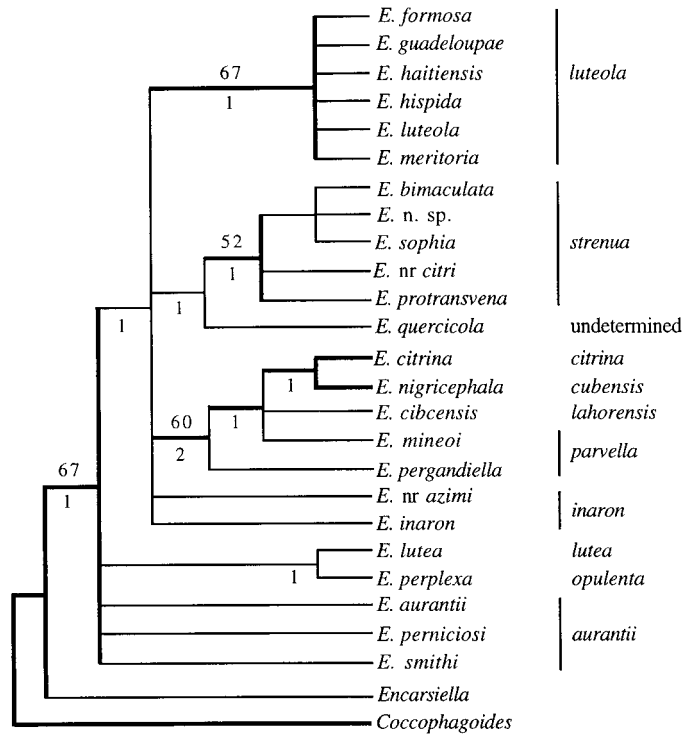


FIG. 21. Strict consensus of four trees from morphological analysis based on successive approximation character weighting (SAW) of 50 most-parsimonious trees. Length 27, CI 0.56, RI 0.79. Thick lines indicate branches supported in both unweighted and weighted (SAW) MP analyses. Bootstrap proportions greater than 50% are shown above each node, with Bremer support shown below. Species groups are as indicated.

RESULTS

Phylogenetic Relationships—Morphology

Analysis of the morphological data generated 50 most-parsimonious trees (length 27, CI 0.56, RI 0.79). Successive weighting reduced this set to 14 of the original 50 trees (same length). The consensus of the SAW trees is illustrated in Fig. 21. Of the previously recognized groups, only the *strenua* and *luteola* groups are found in both parsimony and SAW analyses (thick lines, Fig. 21). The *strenua* group is characterized by close placement of scutellar sensilla (character 8: state 1) and the marginal costal cell seta (11: 1). *Encarsia quercicola* (undetermined placement) also has the closely placed sensilla and is placed as the sister taxon to the *strenua* group only in the SAW analysis. Species in the *luteola* group all share a reduction of segmentation in the mid-tarsus from five to four segments (9: 4). The only other species included in this study with four-segmented mid-tarsi is *E. nigricephala* Dozier (*cubensis* group), but it grouped with four other taxa sharing asetose wing spots (12: 1) and two setae on metasomal tergite 7 (14: 1) and with three of these species that have reduced numbers of setae on the

mesoscutum (6: 2). The grouping of *E. nigricephala* and *E. citrina* is based on a reversal to a three-segmented clava in the female (4: 3). The *parvella* group (*E. pergandiella* Howard and *E. mineoi*) is paraphyletic, and the *aurantii* (*E. aurantii, E. smithi,* and *E. perniciosi*) and *inaron* (*E.* nr *azimi* Hayat and *E. inaron* (Walker)) groups are unresolved. Branches recovered in the parsimony analysis are marginally supported by bootstrap values, as indicated in Fig. 21. Bremer support is generally low for all resolved nodes.

Encarsiella was supported as the sister group of *Encarsia,* but support was weak and based on reduction in number of maxillary palpomeres (from two to one) and fewer setae on the mesoscutal midlobe (2–18 setae) (cf. Fig. 25). However, two palpomeres have been reported for species of *Encarsia* not included in this study (Polaszek and Hayat, 1992), and the number of mesoscutal setae in *Coccophagoides* ranges from 12 to 20 (Hayat, 1998), which together would negate the two *Encarsia* synapomorphies resulting from this analysis. The compression of cellulate sculpture on the scutellum, regarded as the only synapomorphy for *Encarsia* (Polaszek and Hayat, 1992), also occurs in *Coccophagoides,* and thus absence of the trait was treated here as simply an autapomorphy of *Encarsiella* (this would not be autapomorphic if more outgroup genera were included). Even the number of submarginal setae (character 10) can be three or more within *Encarsia,* breaking down any resolution of the *Encarsiella* + *Encarsia* clade.

Phylogenetic Relationships—28S-D2

Complete data set. The complete molecular data set includes a total of 67 *Encarsia* sequences, 2 *Encarsiella noyesi* sequences, and 1 *Coccophagoides fuscipennis* sequence. The aligned data set was 626 bases long with sequences of *Encarsia* (including *Encarsiella*) ranging from 587 to 604 bases; the *Coccophagoides* sequence was 573 bases. Parsimony analysis of the 28S-D2 data for all populations (70 taxa) resulted in four most-parsimonious trees (length 802, CI 0.49, RI 0.84). Successive weighting helped to resolve relationships among species in the *luteola* group by selecting two of the four trees. One of the two SAW trees is presented in Fig. 22; only minor differences between populations of *E. lutea* (Masi) occur in the two SAW trees. The other two MP trees included a different placement of *E. haitensis* as the sister group of *E. guadeloupae,* rather than *E. meritoria* Gahan. These different hypotheses of relationship are reproduced in other analyses discussed later, but overall do not affect our interpretations of species group relationships. Importantly, the gene region supports a clustering of different individuals or populations of each species, even when considerable sequence divergence is present, as in *E. smithi* and *E.* nr *azimi.* Bootstrap values and decay indices (Bremer support) are generally high for apical clades and supported all clades retrieved in both weighted and unweighted analyses.

The single ML tree had a -ln likelihood score of 4750.97 (substitution rate parameters: A–C = 1.00, A–G = 2.23, A–T = 1.00, C–G = 1.00, C–T = 5.67, G–T = 1.00; base frequencies: A = 0.24, C = 0.20, G = 0.26, T = 0.29; proportion of invariant sites [I] 0.28; Γ 0.52). For most relationships, the ML tree was the same as that for parsimony (Fig. 22), with the only major changes being a shift of *E. quercicola* to the base of the tree as the outgroup of the remaining *Encarsia* and *Encarsiella* and a rearrangement of the basal branches (Fig. 22, asterisks) such that the *strenua* group becomes a terminal clade. A minor rearrangement occurs in the *strenua* group (*E. bimaculata* and *E. protransvena* as sister taxa), and the relationships in the *luteola* group are fully resolved as presented in Fig. 22 (one of the two SAW trees). Using parsimony criteria, the ML tree was eight steps longer than any of the MP trees.

Few sequence differences between strains of species sampled multiple times were found. For the 28S-D2 gene region, the average amount of sequence variation within a species was 0.4%, whereas the amount of variation between species was 28-fold greater, with an average of 11.5% (Table 2). The impact of this difference is reinforced when one considers the range of pairwise comparisons. For within-species comparisons, pairwise differences range from 0.0 to 2.4%, whereas between species, the range is 2.0–19.2%. These ranges slightly overlap as shown; however, if the pairwise comparison between the two *E. smithi* strains (2.4%) is ignored, the ranges no longer overlap and it becomes quite easy to demarcate the amount of genetic divergence associated with species-level differences in this genus. It is possible that the two *E. smithi* strains from Australia and Oahu are different but closely related species. The sequences of *E. mineoi* and *Encarsiella* differ by 11%, whereas *E. mineoi* has diverged 15% from all three strains of *E. pergandiella,* which presumably belong to the same *parvella* group; the maximum divergence within the *citrina–pergandiella* clade (Fig. 22) is 11%.

Reduced data set. Analysis of the reduced 28S-D2 data for species (26 taxa) resulted in one most-parsimonious tree (Fig. 23A; length 749, CI 0.50, RI 0.55), which was stable to successive approximations. Character fit to these trees is relatively low, as indicated by the low retention index; however, no other islands were found after an additional 300 random sequence replicates. The MP tree had the same topology as that recovered from the analysis of the complete data set, except with the relationships of the *luteola* group resolved and the same as one of the four MP trees of the complete data set. Bootstrap and Bremer support were comparable for the same nodes in both the complete

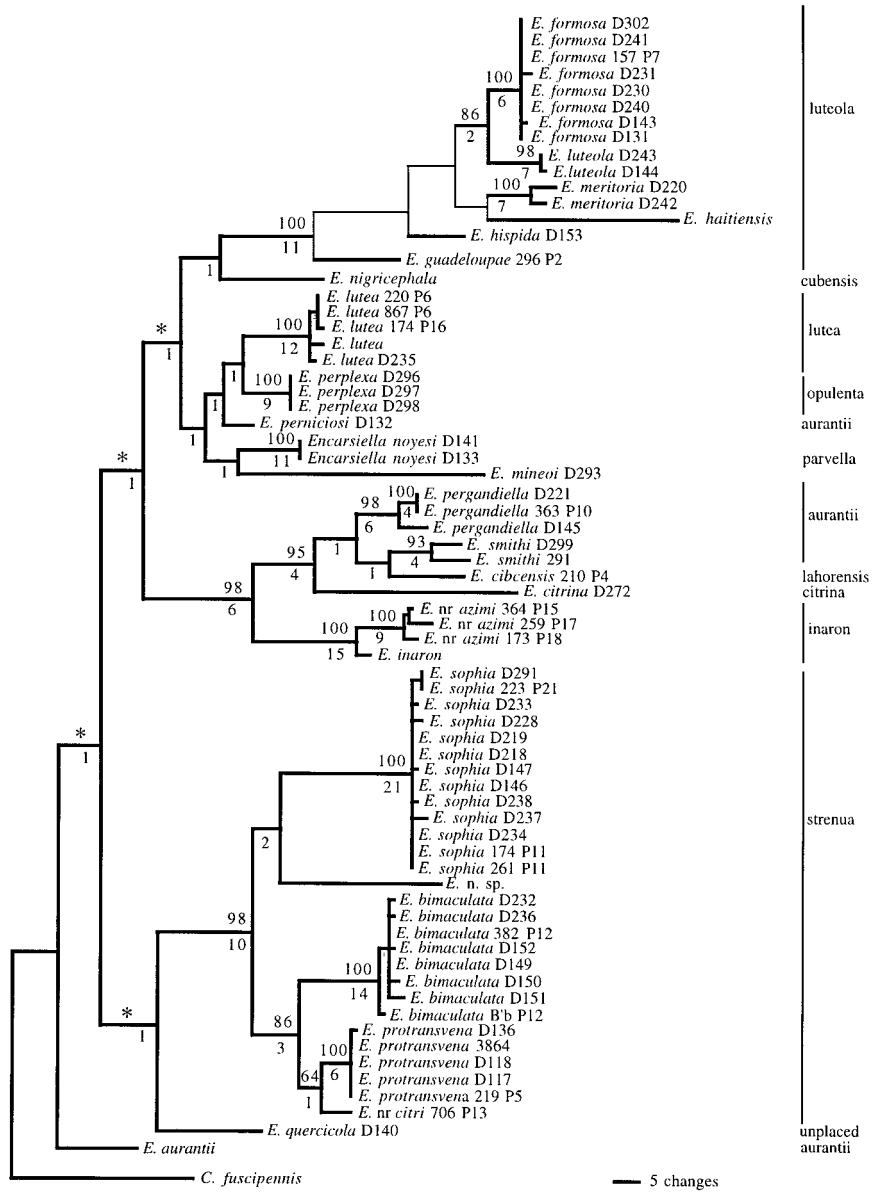


FIG. 22. One of the two SAW trees selected from the four most-parsimonious trees. Length 802, CI 0.49, RI 0.84. Bootstrap values greater than 50% are shown above branches, with Bremer support shown below.

and the reduced analyses, except for bootstrap support for the *E. formosa–E. meritoria* clade within the *luteola* group (72%) and the *E. sophia–E.* n. sp. clade (50%).

TABLE 2

Genetic Distances within and between Species of *Encarsia*

	Within species	Between species
Average	0.004	0.115
Range	0.0–0.24	0.020–0.192
N	155	1961

Note. N, number of pairwise comparisons.

Bremer support for basal nodes was at least 2, except for the *E. nigricephala* + *luteola* group clade.

The single ML tree had a -ln likelihood score of 4328.07 (substitution rate parameters: A–C = 1.0, A–G = 2.19, A–T = 1.0, C–G = 1.0, C–T = 5.93, G–T = 1.00; base frequencies: A = 0.24, C = 0.20, G = 0.26, T = 0.29; proportion of invariant sites [I] 0.30; Γ 0.50). This tree was the same as the MP tree (Fig. 23A), but with only minor rearrangements of species in the *strenua* group and the *luteola* group that match the ML complete analysis. The basal relationships, including a monophyletic *E. quercicola* + *strenua* group, are the same as those for all MP analyses. Using parsimony criteria, the ML tree was four steps longer than the MP

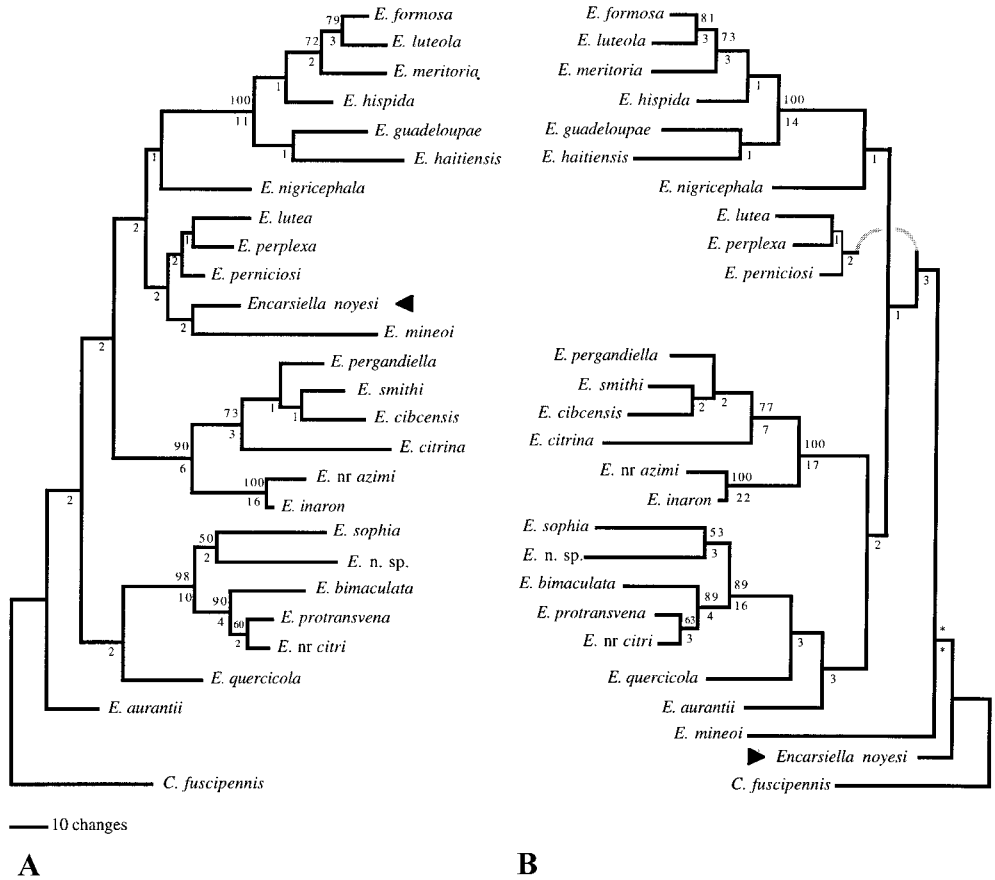


FIG. 23. (A) Single most-parsimonious unconstrained tree based on 28S-D2 rDNA. Length 749, CI 0.50, RI 0.55. (B) One of two most-parsimonious trees based on 28S-D2 rDNA with *Encarsia* constrained as monophyletic; thick lines indicate branches supported in both trees. Length 753, CI 0.49, RI 0.54. Bootstrap values greater than 50% are shown above branches, with Bremer support shown below. Triangle oriented to *Encarsiella*.

tree. In both ML and MP analyses, *Encarsiella noyesi* was again found as a derived taxon within *Encarsia*.

Reduced data set—Encarsia constrained. *Encarsiella* is morphologically very distinct from *Encarsia* (Polaszek and Hayat, 1992). Therefore, we considered it important to test relationships under a hypothesis of *Encarsia* monophyly. *Encarsiella* was forced to be an outgroup by enforcing a constraint tree in which *Encarsia* was monophyletic. Analysis of the reduced data set resulted in two most-parsimonious trees (length 753, CI 0.49, RI 0.54). One of the two trees, which supports a monophyletic *E. lutea* + *E. perplexa* (as favored by SAW) is presented (Fig. 23B). The constrained tree is 4 steps longer than the unconstrained tree. Although differing in basal topology, the MP trees resulting from constrained and unconstrained analyses were not significantly different (Kishino–Hasagawa test, $P = 0.42$; Templeton test, $P = 0.54$; Compare2 T-PTP test, $P = 0.40$). Major differences from the unconstrained analysis include the shift of *E. mineoi* and the *perniciosi* clade to a more basal position within *Encarsia* and the shift of *E. aurantii* to the sister group of the *strenua* + *quercicola* group. Interestingly, except for the relationships which differ between *E. lutea* and *E. perplexa*, the relationships within *Encarsia* are identical to the unconstrained tree except for a reorganization that reflects rerooting the tree to *Encarsiella;* thus, the species relationships within *Encarsia* appear robust.

Phylogenetic Relationships—Combined Data

Reduced data set. The combined analysis included 26 taxa, 626 molecular characters of which 165 were parsimony informative, and 14 morphological characters of which 12 were parsimony informative. The partition homogeneity test was not significant ($P = 0.20$), suggesting that these partitions are not heterogeneous with respect to one another; therefore, data could be combined and analyzed simultaneously. A single parsimonious tree resulted from the combined data (length 790, CI 0.49, RI 0.55); it was identical to one of the reduced molecular unconstrained MP trees (Fig. 23A), except for a switch in the arrangment of *E. citrina* and *E. smithi*, such that *E. smithi* was basal to the clade of species with a bare wing patch (12: 1; Fig. 24) and possibly a narrow forewing (13: 1). This relation-

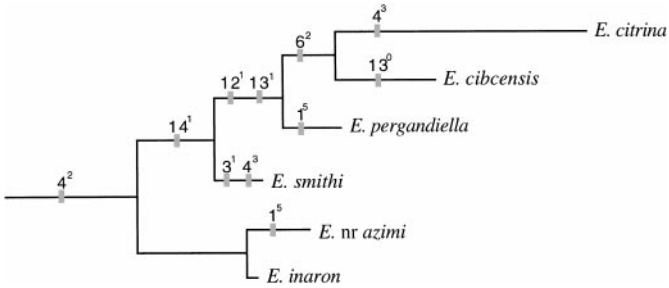


FIG. 24. *Citrina* clade as represented on the single most-parsimonious tree based on the combined unconstrained data set. Length 790, CI 0.49, RI 0.55. Character state changes are mapped onto the relevant branches with derived states denoted as superscript values. Grey bars represent homoplastic character changes across the entire resulting tree.

ship was not found in any of the MP or ML analyses of the molecular data alone. The MP analysis was not stable to SAW, resulting in a single tree 3 steps longer because of a new relationship between *E. bimaculata* and *E. protransvena*, the same as in the ML analysis. The incongruence length difference (ILD) (Farris, 1995) for the MP tree was 14, with 13 of the extra steps introduced by inferring additional homoplasy in the morphological data.

Reduced data set—Encarsia constrained. A single most-parsimonious tree (Fig. 25) resulted from the combined data with *Encarsia* constrained as monophyletic (length 794, CI 0.49, RI 0.54), and this was identical to the reduced molecular constrained tree. The tree was not stable to SAW and again was 3 steps longer for the same reasons cited above. Bremer and bootstrap support values (not reported) were generally the same as those in Fig. 23A, with one notable excep-

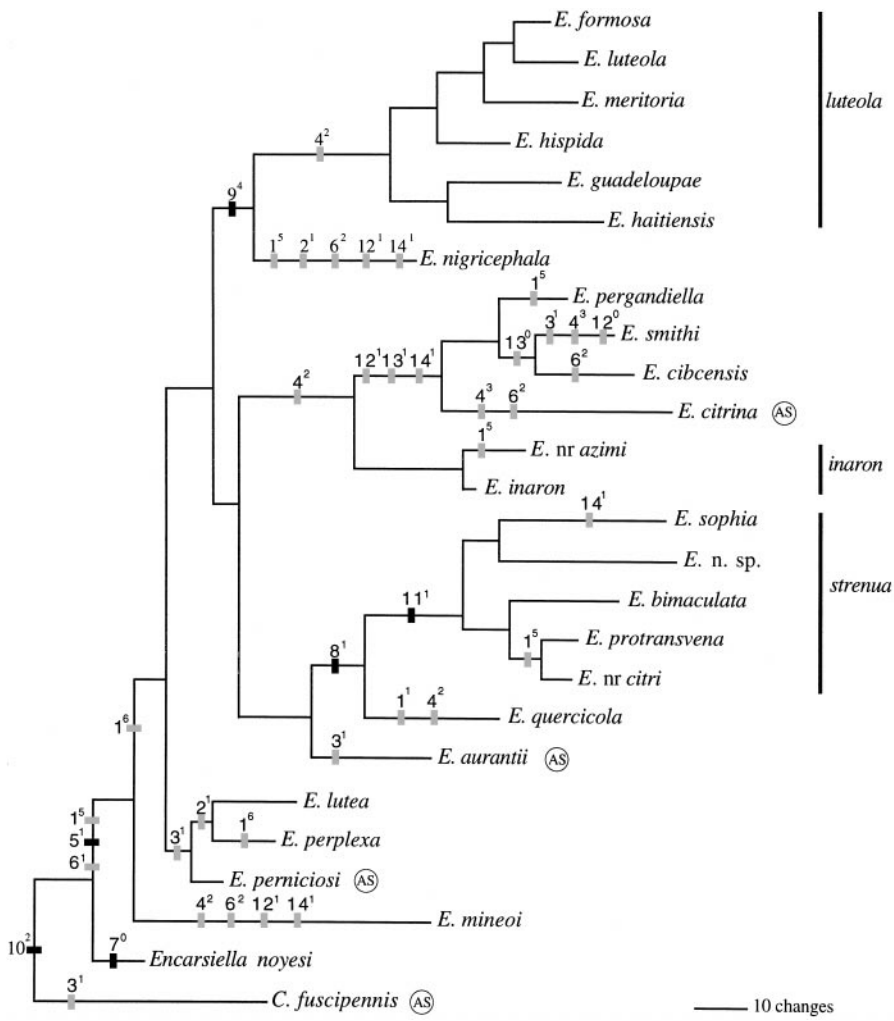


FIG. 25. Single most-parsimonious tree based on the combined data set with *Encarsia* constrained as monophyletic. Length 794, CI 0.49, RI 0.54. Character state changes are mapped onto the relevant branches with derived states denoted as superscript values. Grey bars represent homoplastic character changes. Black bars represent unambiguous character changes. Armored scale (AS) parasites are indicated.

TABLE 3

Support for Monophyly (Indicated by X) of Species Groups, Select Morphological Characters, and Host Choice According to Analysis Performed

	Analysis[a]						
	MO	MP	MLF	MLR	MPRC	CR	CRC
aurantii							
inaron		X	X	X	X	X	X
luteola	X	X	X	X	X	X	X
parvella							
strenua	X	X	X	X	X	X	X
nigricephala + *luteola*		X	X	X	X	X	X
quercicola + *strenua*		X		X	X	X	X
Four-segmented tarsi			X	X	X	X	X
Asetose wing spot	X						
Hard scale parasitism							

[a] Analyses as follows: (MO) morphology only, maximum-parsimony (Fig. 21); (MP) molecular only, maximum-parsimony, both complete (Fig. 22) and reduced (Fig. 23A) data sets; (MLF) molecular only, maximum-likelihood, complete data set; (MLR) molecular only, maximum-likelihood, reduced data set; (MPRC) molecular only, maximum-parsimony, reduced data set, *Encarsia* constraint (Fig. 23B); (CR) combined data, maximum-parsimony, not shown; (CRC) combined data, maximum-parsimony, *Encarsia* constraint (Fig. 24).

tion in the *perniciosi* clade, in which the bootstrap value increased to 69% and the Bremer value to 1. Although differing in basal topology, the single MP trees resulting from each of the constrained and unconstrained analyses were not significantly different (Kishino–Hasagawa test, $P = 0.42$; Templeton test, $P = 0.54$; Compare2 T-PTP test, $P = 0.40$). The ILD was 18, with 14 of the extra steps introduced by inferring additional homoplasy in the morphological data.

Phylogenetic Relationships—Summary

In none of the above analyses of molecular data is *Encarsia* monophyletic, and *Encarsiella* is always placed as the sister group of *E. mineoi*. If one assumes that *Encarsiella* does not render *Encarsia* paraphyletic (morphologically it is very distinct), we consider the best current hypothesis of relationships to be from the combined molecular and morphological analysis, as presented in Fig. 25. The relationships of groups and included species is consistent with most of the other analyses and thus presents the best-corroborated hypothesis for evaluating morphological change. However, if we accept the unconstrained combined hypothesis (Fig. 24) and the nonmonophyly of *Encarsia*, only two traits (bare wing patch and number of mesoscutal setae) would be interpreted differently.

The *strenua* group was monophyletic, as supported by both molecular and morphological characters (Table 3, Fig. 25). Monophyly of the *E. quercicola* + *strenua* group clade was supported only in analyses that included molecular data (except the Tamura–Nei analysis of the complete data set), but without bootstrap support and with Bremer values of 2-3. Morphologically, this clade is justified by the closely spaced scutellar sensilla (8: 1).

The *luteola* group is shown in all analyses to be monophyletic, with strong bootstrap and Bremer support. Within the *luteola* group, *Encarsia formosa* Gahan and *E. luteola* Howard are consistently placed together, which is expected, as these two species are extremely similar and difficult to distinguish using morphology (Babcock and Heraty, 2000). Beyond this, relationships are unsubstantiated. However, none of the analyses showed a monophyletic *E. hispida* + *E. meritoria*, which have only recently been placed into synonymy (Viggiani, 1989) and then subsequently removed from synonymy (Polaszek *et al.*, 1992). The *E. nigricephala* + *luteola* clade is found in all analyses that included molecular data (Table 3), although without bootstrap support and a Bremer value of 1. It also is supported by a single morphological character (reduction in number of tarsi; 9: 4).

The *aurantii* group (sensu Hayat 1998) was not supported in any of the analyses for the three species represented in this analysis. Notably, *E. smithi* had been placed into its own species group (*smithi*) by Viggiani and Mazzone (1979). Huang and Polaszek (1998) did not assign a species group (placement uncertain), and A. Polaszek (personal communication) does not feel that *E. smithi* has anything in common with other species placed into the *aurantii* group.

The *inaron* group was supported in all of the molecular-based analyses, but lacked any morphological synapomorphies (Fig. 25).

A grouping of *E. perniciosi* (*aurantii* group), *E. lutea* (*lutea* group), and *E. perplexa* (*opulenta* group) was supported in all results that included molecular data, with *E. lutea* and *E. perplexa* most often treated as sister taxa; however, bootstrap support (69%) was

present only in the combined analysis. A single morphological character (compressed basal flagellomere in females; 3: 1) supported this clade.

In all of the analyses, *Encarsia citrina* (*citrina* group), *E. pergandiella* (*parvella* group), and *E. cibcensis* (*lahorensis* or *perflava* group), each with a bare wing patch, are placed into a clade with *E. smithi* (*aurantii* group) (Figs. 22, 23A, 23B, and 25), with *citrina* basal in all analyses except the combined unconstrained analysis (Fig. 24). All four taxa have only two medial setae on metasomal tergite 7, a feature otherwise found only in *E. sophia* of the *strenua* group. The *inaron* group and the *inaron* + *E. citrina* clade were both strongly supported by bootstrap and Bremer support in all analyses. In all cases, species represented by more than one population grouped together, even when the sequence divergence between populations was relatively high, such as in *E. smithi.* Sequence divergence within species was usually negligible, even when populations were sampled from different continents (i.e., *E. bimaculata, E. formosa,* and *E. sophia*).

Monophyly of the *parvella* group was not supported in any of the molecular analyses. As well as sharing apomorphic traits, including the bare wing patch, reduction in number of setae on Mt_7, and possibly a narrowed wing, *Encarsia mineoi* is very similar morphologically to *E. pergandiella* and we find it surprising that they would not form a group. However, a sequence divergence of 15% is extreme compared to other closely related species (as placed in the analysis). *E. mineoi* alone is not affecting placement of *Encarsiella,* as its removal does not alter the results. The sequence of *E. mineoi* was compared with two other individuals from the same population, and we are confident of both the sequence and the alignment. Similar long branches in *E. citrina (citrina), E. haitensis (luteola),* and *E.* n.sp. (*strenua*) had no effect on their respective group placement.

DISCUSSION

In general, the different analyses of molecular and combined data were all in agreement for major groups within *Encarsia,* with consistent support for monophyly of the *luteola, strenua,* and *inaron* groups. The relationships of species within these remained unchanged in almost all of the analyses. In all unconstrained analyses using molecular data, *Encarsiella* was included within *Encarsia,* rendering the latter genus paraphyletic. However, when *Encarsia* was constrained to be monophyletic, the branching pattern was identical, although inverted to match the new *Encarsiella* root. Trees resulting from the analysis of 26 taxa based only on morphology were poorly resolved, although the *strenua* and *luteola* groups were supported.

An important aspect of this study was the evaluation of what appeared to be, for the most part, rather trivial character state changes. *Encarsia* are minute wasps that are relatively uniform in morphology, with most character changes involving the reduction or loss of particular traits or the acquisition of very minor characters, such as the apical costal setae of the *strenua* group. Another problem is the simple evaluation of polarity change. For example, if the costal cell seta were primitive for *Encarsia,* then its absence may be a defining character for the other species. Obviously these changes can be evaluated with outgroup analysis, but closely related genera within Coccophaginae are undergoing similar reductive changes and considerable homoplasy exists for traits both within the subfamily and within *Encarsia.*

Only three morphological characters change unambiguously within *Encarsia* (Fig. 24, black bars): close placement of scutellar sensilla (character 8: state 1), mid-tarsus with four tarsomeres (9: 4), and presence of one to three marginal setae in the costal cell (11: 1). All 40 species of the *strenua* group have the costal cell seta, and as far as we know this occurs nowhere else within *Encarsia.* The close placement of sensilla appears to be a unique event within *Encarsia,* supporting a monophyletic *E. quercicola* + *strenua* group; however, we have not included representatives of the *citrella* group and *Encarsia bella* (Gahan), which have a similar placement of the sensilla. The fusion of apical tarsomeres on the mid-tarsus supports monophyly of the *cubensis* and *luteola* groups, with the implication that this is a unique evolutionary event within *Encarsia* rather than a series of independent changes within various species groups, as proposed by Hayat (1998). A four-segmented tarsus is also found in *E. moyhuddini* (unplaced group) and in the *singularis* group (Huang and Polaszek, 1998), but unfortunately these were not available for study. The only other potential unambiguous character for *Encarsia* was the reduction to a single maxillary palpomere (5: 1), but this synapomorphy was based only on species in this data set, and two-segmented palpi have been recognized for 2 species by Polaszek and Hayat (1992); this trait needs to be thoroughly assessed in all *Encarsia* and other outgroup genera to determine its overall value. Alone, it is probably a poor defining character for the genus.

The remaining character state changes are homoplastic within *Encarsia.* The bare wing patch (character 12: 1) is proposed to have evolved four times and undergone one reversal (Fig. 24). This character, along with a change to two setae on metasomal tergite 7 (14: 1), supports the monophyly of a clade that includes representatives of four species groups: *E. pergandiella* (*parvella*), *E. smithi* (*aurantii*), *E. cibcensis* (*lahorensis* or *perflava*), and *E. citrina* (*citrina*). The *parvella* and *citrina* groups both have been treated as distinct genera, *Aleurodiphilus* and *Aspidiotiphagus,* respectively

(DeBach and Rose, 1981); however, their placement within this clade, nestled within what could be regarded as more typical *Encarsia,* would not support this proposal. *E. smithi* is the only member of the *citrina* clade without a bare wing patch, although there are fewer setae around the stigmal vein (Fig. 15) than found in species with a completely setose wing (not shown). Also, its affiliations with the *aurantii* group are uncertain (Huang and Polaszek, 1998). Prior to the reassignment by Hayat (1998), this species was placed in its own species group, and the current assignment in the *aurantii* group is not accepted by A. Polaszek (personal communication).

Differential support for clades or individuals by characters that appear to perform poorly on the overall tree can offer the only morphological support for some of the more robust clades resulting from the molecular analysis. For example, the only character supporting the *luteola* group and the *inaron* + *citrina*-clade group is the two-segmented female clava (4: 2), even though this character change is derived three other times on the tree and undergoes two reversals within the *citrina* clade (character CI 0.17, character RI 0.54). The sensorial complex on the male antenna (CI and RI 0.5) is considered to be derived independently in the *cubensis* and the *lutea* + *opulenta* groups. The sensillar structure on the second flagellomere of *E. nigricephala* is morphologically very different from that found in the *lutea* and *opulenta* groups (cf. Viggiani and Battaglia, 1984; Viggiani and Laudonia, 1989; Viggiani, 1996), and new separate coding as two different states would be justified. Also, we initially chose not to code the peculiar sensillar patch on the antenna of *E. perniciosi* as a derived structure; however, the placement of *E. perniciosi* as sister to *lutea* + *opulenta* would suggest that it is the same feature and further that it could be a precursor to the more derived glandular structure. Last, the most poorly performing morphological character, fusion of the apical flagellomeres in males (1: 5; character CI and RI 0.12), was autapomorphic for three species, but did support the relationship between *E. protransvena* and *E.* nr *citri* within the *strenua* group. Notably, this grouping was rejected in all ML analyses.

Although only species parasitic on armored acales or whiteflies were included in the analysis, the resulting trees can provide a hypothesis for the shift in parasitism between the two groups. The outgroup taxa are equivocal for host association, with *Coccophagoides* parasitic on Diaspididae and *Encarsiella* on Aleyrodidae. On the constrained tree (Fig. 25), whitefly parasitism is ancestral for *Encarsia,* with three independent shifts to parasitism of armored scales in *E. perniciosi, E. aurantii,* and *E. citrina.* Notably, *E. aurantii* and *E. perniciosi* are considered to be closely related by A. Polaszek (personal communication). In the unconstrained trees (Fig. 23A), armored scale parasitism would be basal for *Encarsia* (present in *E. aurantii* and *Coccophagoides*), with a shift to whitefly parasitism in most species, and then two independent associations with armored scales in *E. perniciosi* and *E. citrina.* In either scenario, the evidence suggests multiple shifts to parasitism of armored scale and a single shift to whitefly hosts either within or outside of *Encarsia.*

This study is truly preliminary, as it is based on only 26 species from a genus that could eventually include more than 300. However, the 28S-D2 sequence data provide an initial hypothesis of relationships for *Encarsia* that has allowed testing the evolution of seemingly trivial character state changes and assessing their value for supporting clades within *Encarsia.* The addition of morphological data to the constrained data set favored one of the two hypotheses resulting from use of molecular data alone. Morphological data alone would never achieve a similar degree of resolution among species and groups. Including the molecular data allowed for better interpretation of character state change and the utility of certain characters to support nodes, thus improving our ability to assess group relationships and classify species known only from museum specimens. Our study represents only a cursory look at a very complex and diverse assemblage. Yet the results are encouraging and with more taxa the group associations will become more resolved and questions regarding behavioral changes can be addressed.

APPENDIX 1

Species of *Encarsia* Used According to Species Group

Species group Species	Strain identifier	Collection location
aurantii group		
E. aurantii[a]*	H97-D77	Texas
E. perniciosi[b]*	D132	California (UCR culture)
E. smithi[c]*	D299	Hawaii: Makakilo, Oahu Is.
E. smithi[d]	291a P24	Micronesia: FSM Truk
citrina group (= *Astidiotiphagus*)		
E. citrina[b]*	D272	California: Riverside Co.
cubensis group		
E. nigricephala[d]*	218	French Polynesia: Tahiti, Punaalu
inaron group		
E. nr *azimi*[d]	173 P18	Australia: WA, Kununurra
E. nr *azimi*[d]	259 P17	Australia: QLD, Ayr
E. nr *azimi*[d]*	364 P15	Australia: SA, Renmark
E. inaron[a]*		New Zealand (culture)
lahorensis group		
E. cibcensis[d]*	210 P4	Cook Islands: Raratonga
lutea group		
E. lutea[a]*	M94-129	California (culture)
E. lutea[d]	174 P16	Australia: WA, Wanneroo
E. lutea[d]	220 P6	Cook Islands: Raratonga
E. lutea[d]	867 P6	Tonga
E. lutea[e]	D235	Cyprus (M93064)
luteola group		
E. formosa[d]	157 P7	Australia: ACT, Canberra
E. formosa[f]*	D131	California (UCR colony)
E. formosa[g]	D143	UK: Bunting (company)
E. formosa[e]	D230	Greece (M92017)
E. formosa[e]	D231	Egypt (M92030)
E. formosa[h]	D240	UK: original stock 1932
E. formosa[i]	D241	UK: original stock 1928
E. formosa[j]	D302	Maryland: Beltsville strain
E. guadeloupae[d]*	96 P22	Nauru
E. haitiensis[d]*		Australia: QLD, Cairns
E. hispida[k]*	D153	California: San Diego
E. luteola[g]*	D144	California: Brawley
E. luteola[l]	D243	California: Imperial Co.
E. meritoria[m]*	D220	California: Riverside Co.
E. meritoria[l]	D242	California: Imperial Co.
opulenta group		
E. perplexa[c]*	D296	Guatemala: Coatepeque
E. perplexa[c]	D297	Hawaii: Oahu Is., Makakilo
E. perplexa[c]	D298	Hawaii: Oahu Is., Ala Wai
parvella group (= *Aleurodiphilus*)		
E. mineoi[c]*	D293	Egypt: Cairo
E. pergandiella[d]	363 P10	Australia: QLD, Darling Downs
E. pergandiella[g]	D145	Brazil (M94055)
E. pergandiella[n]*	D221	California, Riverside

APPENDIX 1—*Continued*

Species group Species	Strain identifier	Collection location
strenua group		
E. bimaculata[d]	382 P12	Australia: NT, Darwin
E. bimaculata[d]	B′b P12	Australia: QLD, Bundaberg
E. bimaculata[o]	D149	Guatemala (Florida culture)
E. bimaculata[o]	D150	India (Florida culture)
E. bimaculata[o]*	D151	Israel (Florida culture)
E. bimaculata[o]	D152	Sudan (Florida culture)
E. bimaculata[e]	D232	India (M92018)
E. bimaculata[e]	D236	Taiwan (M94016)
E. nr *citri*[d]*	706 P13	Australia: QLD, Munduberra
E. n. sp.[d]		Australia: QLD, Oakey
E. protransvena[p]	3864	Florida
E. protransvena[d]	219 P5	Tahiti: Paea
E. protransvena[f]	D117	California: (UCR culture)
E. protransvena[f]	D118	California: (UCR culture)
*E. protransvena**	D136	California: Orange County
E. sophia[d]	174 P11	Australia: WA, Wanneroo
E. sophia[d]	223 P21	Tahiti: Papona
E. sophia[d]	261 P11	Australia: QLD, Ayr
E. sophia[g]	D146	Spain (M3003)
E. sophia[g]	D147	Florida
E. sophia[q]	D218	Ethiopia
E. sophia[r]	D219	Florida
E. sophia[s]	D228	Pakistan (M95107)
E. sophia[e]	D233	India (M93002)
E. sophia[e]	D234	Spain (M93003)
E. sophia[e]*	D237	Thailand (M94041)
E. sophia[e]	D238	Malaysia (M94047)
E. sophia[c]	D291	Hawaii: Oahu Is., Waimanalo
Unplaced		
*E. quercicola**	D140	California: San Bernardino Co.

Note. Contributor of specimen is indicated by superscript.
* Sequence used in 28S-D2 subset analysis and in combined data analysis.
[a] Sequence provided by B. Campbell, USDA, Albany, CA.
[b] B. Luck, UC Riverside.
[c] M. Ramadan and W. Nagamine, Hawaii Department of Agriculture.
[d] Collected, extracted, and sequenced by Australian Whitefly Project, CSIRO, Australia.
[e] D. Vacek and R. Ruiz, USDA/APHIS/PPQ, Mission Plant Protection Center, Mission, TX.
[f] T. Bellows, UC Riverside.
[g] M. Hunter, University of Arizona.
[h] The Green Spot, LTD, Nottingham NH.
[i] Applied Bionomics, Sidney, B.C., CAN.
[j] J. Sanderson, Cornell University.
[k] M. Rose, Montana State University.
[l] W. Roltsch, California Department of Food and Agriculture.
[m] M. Guillen, UC Riverside.
[n] T. Pinchard, UC Riverside.
[o] R. Nguyen, University of Florida.
[p] L. Ehler, UC Davis.
[q] D. Gerling, Tel Aviv University.
[r] H. McAuslane, University of Florida.
[s] M. Ciomperlik, USDA/APHIS/PPQ, Mission, TX.

APPENDIX 2

Morphological Matrix

	Characters													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Coccophagoides	6	0	1	3	2	0	1	0	5	3	0	0	0	0
Encarsiella	6	0	0	3	2	0	0	0	5	2	0	0	0	0
E. aurantii	6	0	1	3	1	1	1	0	5	2	0	0	0	0
E. perniciosi	5	0	1	3	1	1	1	0	5	2	0	0	0	0
E. smithi	6	0	1	3	1	1	1	0	5	2	0	0	0	1
E. citrina	?	?	0	3	1	2	1	0	5	2	0	1	1	1
E. nigricephala	5	1	0	3	1	2	1	0	4	2	0	1	0	1
E. nr *azimi*	5	0	0	2	1	1	1	0	5	2	0	0	0	0
E. inaron	6	0	0	2	1	1	1	0	5	2	0	0	0	0
E. cibcensis	6	0	0	2	1	2	1	0	5	2	0	1	0	1
E. lutea	5	1	1	3	1	1	1	0	5	2	0	0	0	0
E. formosa	6	0	0	2	1	1	1	0	4	2	0	0	0	0
E. guadeloupae	?	?	0	2	1	1	1	0	4	2	0	0	0	0
E. haitiensis	?	?	0	2	1	1	1	0	4	2	0	0	0	0
E. hispida	6	0	0	2	1	1	1	0	4	2	0	0	0	0
E. luteola	6	0	0	2	1	1	1	0	4	2	0	0	0	0
E. meritoria	6	0	0	2	1	1	1	0	4	2	0	0	0	0
E. perplexa	6	1	1	3	1	1	1	0	5	2	0	0	0	0
E. mineoi	5	0	0	2	1	2	1	0	5	2	0	1	0	1
E. pergandiella	5	0	0	2	1	1	1	0	5	2	0	1	1	1
E. bimaculata	6	0	0	3	1	1	1	1	5	2	1	0	0	0
E. nr *citri*	5	0	0	3	1	1	1	1	5	2	1	0	0	0
E. n. sp.	6	0	0	3	1	1	1	1	5	2	1	0	0	0
E. protransvena	5	0	0	3	1	1	1	1	5	2	1	0	0	0
E. sophia	6	0	0	3	1	1	1	1	5	2	1	0	0	1
E. quercicola	5	0	0	2	1	1	1	1	5	2	0	0	0	0

Note. Characters and states are discussed in the text.

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