A revision of the *Encarsia pergandiella* species complex (Hymenoptera: Aphelinidae) shows cryptic diversity in parasitoids of whitefly pests

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Abstract. *Encarsia pergandiella* Howard, described from North America (USA), and *Encarsia tabacivora* Viggiani, described from South America (Brazil) (Hymenoptera: Aphelinidae), are two formally recognized taxonomic entities, that have been treated by several authors as synonyms due to lack of strong diagnostic characters. Taxonomy of these species is further complicated because several populations, geographically separated and differing in their biology, have been included under the concept of *E. pergandiella*. Among these, a population originally collected in Brazil and introduced to North America reproduces by thelytokous parthenogenesis and is infected by the symbiont *Cardinium*, while a morphologically indistinguishable population, naturally occurring in Texas, is biparental and infected by a related strain of *Cardinium* that induces cytoplasmic incompatibility. A third population known from California and introduced to the Old World is biparental and uninfected by intracellular symbionts. While adult females of the first two populations have entirely light yellow bodies and pupate face up (light form), those of the third population have largely brown bodies and pupate face down (dark form). Other dark form populations are known from Texas, Florida and New York. Because these parasitoids are economically important biological control agents of cosmopolitan whitefly pests, it is critical to characterize them correctly. In this study, we integrated molecular and morphometric analyses to substantiate observed differences in biological traits, and resolve the complicated taxonomy of this species complex. We sequenced the mitochondrial cytochrome *c* oxidase subunit I gene and the D2 region of the ribosomal 28S gene for individuals of both light form (from Texas and Brazil) and dark form (from California, Texas, Italy and Canary Islands) originating from laboratory cultures or collected in the field. Phylogenetic analysis unambiguously distinguished three well-supported groups corresponding to the Texas light form, the Brazil light form and the dark form. Individuals of these three groups, in combination with all available type material (*E. pergandiella*, its synonym *Encarsia versicolor* Girault and *E. tabacivora*) and additional museum specimens of the dark form from New York and Italy, were subjected to multivariate morphometric analyses using Burnaby principal component analysis followed by a linear discriminant analysis, and multivariate ratio analysis. Overall, the analyses showed that: (i) *E. pergandiella* and *E. tabacivora* are two distinct species; (ii) the thelytokous Brazil light form corresponds to *E. tabacivora*; (iii) the biparental Texas light form is a new species formally described

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here as *Encarsia suzannae* **sp.n.**; (iv) two new biparental species can be referred to the dark form, one described as *Encarsia gennaroi* **sp.n.** including the populations sampled in California, Texas, Italy and Canary Islands, and the other corresponding to the population from New York described as *Encarsia marthae* **sp.n.** A dichotomous key for both sexes of the species of the *E. pergandiella* complex is provided for identification.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid: zoobank.org:pub:278475A0-C2C4-4400-A042-A5716457829D.

Introduction

Encarsia Förster is a large and extremely diverse genus of minute wasps in the chalcidoid family Aphelinidae, currently including 438 valid species, mostly parasitoids of white-flies (Hemiptera: Aleyrodidae) and armoured scale insects (Hemiptera: Diaspididae) (Polaszek *et al.*, 2004; Noyes, 2016). Like other Hymenoptera, sexual *Encarsia* species are hap-lodiploid and reproduce by arrhenotokous parthenogenesis, i.e. females develop from diploid, fertilized eggs, whereas males develop parthenogenetically from haploid, unfertilized eggs. Most species of *Encarsia* are also autoparasitoids, with females being primary parasitoids and males hyperparasitoids of larvae of their own or of other species (Hunter & Woolley, 2001). Additionally, several species of *Encarsia* reproduce by thelytokous parthenogenesis (diploid females develop from unfertilized eggs) with rare or unknown males. All thelytokous species of *Encarsia* examined to date have been found to be infected by parthenogenesis-inducing endosymbiotic bacteria, e.g. *Wolbachia* in *Encarsia formosa* Gahan (van Meer *et al.*, 1999; Zchori-Fein *et al.*, 2001) and *Cardinium* in all other examined species (Zchori-Fein *et al.*, 2001, 2004; Giorgini *et al.*, 2009). Further, in some species, these bacterial symbionts cause cytoplasmic incompatibility, a form of reproductive incompatibility occurring when uninfected females mate with infected males, while the other three possible crosses between symbiont-infected and uninfected males and females produce normal numbers of offspring (Hunter *et al.*, 2003; Perlman *et al.*, 2006; Gebiola *et al.*, 2016).

Encarsia is one of the parasitoid genera with the highest number of species used in successful biological control programmes (Heraty, 2004). Due to its economic importance, this genus has attracted considerable attention and has become the focus of different basic and applied research. However, although the taxonomy of some species groups has been clarified, it remains unresolved for many other species groups, leaving the identification of common species still problematic (Heraty *et al.*, 2008). Difficulties arise as a result of the small size of these wasps (<1 mm), few differentiating morphological traits in some groups of species (Heraty & Polaszek, 2000; Manzari *et al.*, 2002; Polaszek *et al.*, 2004), and the existence of cryptic species (Giorgini & Baldanza, 2004; Monti *et al.*, 2005), thus making it difficult to separate species by well-defined characters. *Encarsia pergandiella* Howard and *Encarsia tabacivora* Viggiani in the *parvella* species group are prime examples of *Encarsia* species whose limits are not yet well defined. These two species are important biological control agents of whiteflies, including global pests such as *Bemisia tabaci* (Gennadius) (*sensu lato*), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood).

Encarsia pergandiella was first described from material collected on *Aleyrodes* (probably *Trialeurodes* sp.) in Washington, D.C., USA. The female body colour was described as uniform honey-yellow, with no males or pupal characters reported (Howard, 1907). Girault (1908) later described *Encarsia versicolor* as a parasitoid of *T. vaporariorum* in Illinois, USA, with the body described as 'general color pale cadmium yellow, marked with variable dusky'. However, *E. versicolor* was later synonymized by Gahan (in Peck, 1951) with *E. pergandiella*. In contrast with the previous descriptions, Gerling (1966), Riv-nay & Gerling (1987) and Viggiani (1988) stated that the body colour of females of *E. pergandiella* from California is at least in part brown. De Santis (1981) described a new parasitoid attack-ing *B. tabaci* in Brazil as *Encarsia bemisiae*, whose body was described as predominantly yellow. When Viggiani & Mazzone (1979) synonymized the genus *Prospaltella* Ashmead under *Encarsia* Förster, *E. bemisiae* became preoccupied by *Encarsia bemisiae* (Ishii, 1938). Therefore, Viggiani (1985) renamed the junior secondary homonym as *Encarsia tabacivora*. Polaszek *et al.* (1992) later treated *E. tabacivora* as a synonym of *E. pergandiella* based on the study of morphological features of material from many localities worldwide as well as type material of both species. According to Polaszek *et al.* (1992), *E. pergandiella* displays considerable variation throughout its distribution range, both in coloration and in length of antennal segments. Similarly, following examination of many specimens collected in different areas and on different hosts, Evans (1993) concluded that intermediate forms were present and that the variation seen in *E. pergandiella* was probably host- or environmentally induced. However, biological differ-ences associated with body colour were subsequently found. In particular, one of the authors (R.C.J.) reported that two sympatric and reproductively isolated populations in southern Texas differed in the body colour and pupal orientation: a 'dark form' pupating face down, which she considered to be *E. pergandiella*, and a 'light form' pupating face up, which she identified as *E. tabacivora* (Johnson, 1996). Liu & Stansly (1996b) found a similar difference in pupal orientation between a population of 'dark form' *E. pergandiella* from southern Florida, and *Encarsia* sp. nr *pergandiella* from Brazil that was entirely yellow. The behaviour of pupating face down was also observed in 'dark form' *E. pergandiella* from California (Gerling, 1966; M. Giorgini and P.A. Pedata, unpublished data). More recently, based on Johnson (1996), Hernández-Suárez

et al. (2003) suggested *E. tabacivora* to be a valid species. Myartseva & Evans (2008) revalidated it based on characters such as the number of setae on the sixth tergite, and the relative length of the ovipositor to midtibia, and of antennal segments. However, these authors stated that both species had a light and a dark form. *Encarsia pergandiella* (*sensu* Myartseva & Evans) appears to have a wide geographic distribution, being present in the American continents in both forms (Myartseva & Evans, 2008), as well as in the Old World, with only the dark form present (Viggiani, 1988; Hernández-Suárez *et al.,* 2003). By contrast, *E. tabacivora* (*sensu* Myartseva & Evans) has only been recorded from South America and the southern United States (Myartseva & Evans, 2008). Specimens reported from Australia by Schmidt & Polaszek (2007), who considered *E. pergandiella* and *E. tabacivora* as synonyms, seemed to be identical to European dark specimens based on their illustrations and description. In the New World, the complex *E. pergandiella*/*E. tabacivora* has been reported to be very effective in controlling *B. tabaci* and *T. vaporariorum* (Goolsby *et al.,* 1998; Schuster *et al.,* 1998; Gould *et al.,* 2008). In the Old World, *E. pergandiella* (dark form) established in the Mediterranean Basin after being independently introduced to Italy (Viggiani & Mazzone, 1980; Viggiani, 1988) and Israel (Rivnay & Gerling, 1987) from California in 1979 for classical biological control of *T. vaporariorum* and *B. tabaci*. In southern Italy, this species is considered one of the most effective natural enemies of whitefly pests (Giorgini & Viggiani, 2000; Nannini *et al.,* 2006). More recently, another, probably conspecific, population of *E. pergandiella* from California has been introduced into Egypt (Abd-Rabou, 2006; S. Abd-Rabou, personal communication). That the tangled taxonomic history of *E. pergandiella* may reflect the existence of cryptic species has also come up in studies on reproductive manipulations induced by endosymbiotic bacteria. It has been shown that populations from different geographic areas have specific reproductive mode and endosymbiont infection status. In particular, among the 'light form' populations pupating face up, the one from Texas is biparental and infected by a strain of *Cardinium* causing cytoplasmic incompatibility (Hunter *et al.,* 2003), while the 'light form' population from Brazil reproduces exclusively by thelytokous parthenogenesis and is infected by a different strain of *Cardinium* (Zchori-Fein *et al.,* 2001, 2004). Conversely, the 'dark form' population pupating face down from California and introduced to the Old World reproduces sexually and is uninfected by *Cardinium* or other endosymbiotic bacteria (Zchori-Fein *et al.,* 2001; E. Mann, C. Stouthamer, S.E. Kelly, M. Gebiola, M.S. Hunter and S. Schmitz-Esser, unpublished data).

Understanding the relationships among closely related populations is interesting from the standpoint of systematics and evolutionary biology. Studies of these populations may provide clues to understanding the radiation that resulted in over 400 species in the genus *Encarsia*. In addition, closely related or cryptic species often differ with respect to biological attributes that may affect their efficacy in biological control (DeBach & Rosen, 1991; Beltrà *et al.,* 2015). Further, the correct identification of parasitoid populations is indispensable for risk assessment of biological control agents (Barratt *et al.,* 2010). Species-level

systematics is therefore critical for the successful use of cryptic species for biological control (Heraty, 2004). Integrative taxonomy provides a practical framework for species delimitation by integrating molecular, morphological, morphometric, biological, ecological and behavioural data as independent lines of evidence (Dayrat, 2005; Chesters *et al.,* 2012; Gebiola *et al.,* 2012). The taxonomy and systematics of *Encarsia* have greatly benefited from the use of molecular markers, such as the expansion region D2 of the ribosomal gene 28S (Babcock & Heraty, 2000; Babcock *et al.,* 2001; Manzari *et al.,* 2002) and the mitochondrial gene cytochrome *c* oxidase subunit I (COI) (de León *et al.,* 2010). However, the problem of genetically identifying cryptic species of *Encarsia* has been tackled only twice, using COI alone (Monti *et al.,* 2005) or in combination with 28S-D2 (Gebiola *et al.,* 2016). The application of multivariate morphometrics to revise species groups of *Encarsia* has also been useful (Heraty & Polaszek, 2000; Pedata & Polaszek, 2003; Polaszek *et al.,* 2004), and has enabled the discovery of cryptic species (Manzari *et al.,* 2002; Giorgini & Baldanza, 2004). However, as morphological characters generally scale with body size, interpretation of difference solely in terms of measurements may draw misleading conclusions, due to the existence of size differences both within and among species. Therefore, the treatment of size differences among groups to be compared remains an important issue. However, no standard method is universally accepted to factor out the size effect (Bookstein *et al.,* 1985; Darroch & Mosimann, 1985; Jungers *et al.,* 1995; Berner, 2011). Recently, Baur & Leuenberger (2011) have developed a new statistical approach, the multivariate ratio analysis (MRA), which allows an immediate inference on allometry and effectively identifies diagnostic ratios, thus facilitating the identification of cryptic species (László *et al.,* 2013).

This study aims to characterize the genetic and morphological diversity hidden under the species concepts of *E. pergandiella* and *E. tabacivora* and to resolve the complicated taxonomy of this species complex under an integrative taxonomy perspective. We combined biological and behavioural data with evidence obtained from molecular systematics (sequencing the genes COI and 28S-D2) and from multivariate analyses of morphometric traits, factoring out the size effect using two different approaches, the backprojection method of Burnaby (1966) and the use of ratios by implementing MRA. We ultimately revealed unexpected cryptic diversity and provided a resolved classification for this ecologically and economically important species complex.

Materials and methods

Study material

Laboratory material

Three reproductively isolated populations of the *E. pergandiella* complex were reared in the laboratory. A culture of the dark form with biparental reproduction was started from wasps collected on *T. vaporariorum* in Italy, where this parasitoid was originally introduced from California. The two other cultures
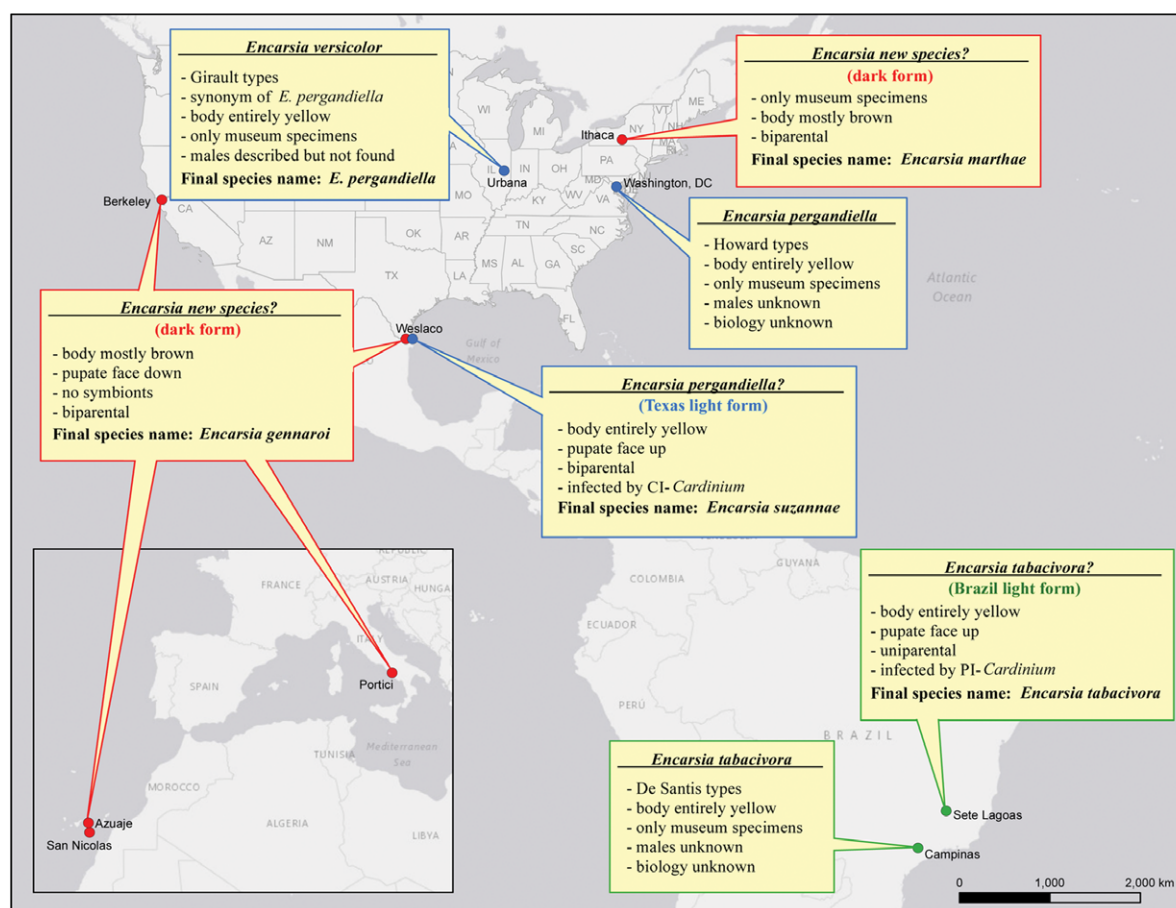
Fig. 1. Populations studied, initial species hypothesis and final species designations for the *Encarsia pergandiella* complex. [Colour figure can be viewed at wileyonlinelibrary.com].

corresponding to the light form were started from wasps collected on *B. tabaci* from Weslaco, Texas (with biparental reproduction), and Sete Lagoas, Minas Gerais, Brazil (with uniparental reproduction). They have both been shown to harbour the bacterial endosymbiont *Cardinium*, which causes different reproductive effects in these hosts: thelytokous parthenogenesis in the Brazil population (Zchori-Fein *et al.,* 2001) and cytoplasmic incompatibility in the Texas population (Hunter *et al.,* 2003). Conversely, the laboratory dark form does not host *Cardinium* or other endosymbiotic bacteria (Zchori-Fein *et al.,* 2001; E. Mann, C. Stouthamer, S.E. Kelly, M. Gebiola, M.S. Hunter and S. Schmitz-Esser, unpublished data). In the laboratory, females of these three cultures were reared either on *T. vaporariorum* infesting bean (*Phaseolus vulgaris* L.) (Fabaceae) or on *B. tabaci* infesting cowpea (*Vigna unguiculata* (L.) Walp.) (Fabaceae), while males of sexual dark and light forms were produced using *Encarsia formosa* Gahan as a secondary host.

Field material

Individuals of both dark and light sexual forms were collected from *B. tabaci* in Weslaco, Texas. Individuals of the dark form were also collected in Portici, Italy, from *T. vaporariorum*

on tomato plants (Solanaceae), in Berkeley, California, from *Trialeurodes* sp. on *Malva* sp. (Malvaceae) and in the Canary Islands, Spain, from *Trialeurodes* sp. on *Lavendula* sp. (Lamiaceae) and *Nicotiana glauca* Graham (Solanaceae) (Fig. 1).

Museum material

Morphometric analyses were also implemented with specimens borrowed from museum collections. Type specimens of *Encarsia pergandiella* Howard (5 syntypes), *Encarsia tabacivora* Viggiani (as *Encarsia bemisiae* De Santis, holotype and 15 paratypes) and *Encarsia versicolor* Girault (9 syntypes) were borrowed from the United States National Museum, Smithsonian Institution, Washington, DC, USA (USNM), from the Museo de La Plata, La Plata, Argentina (UNLP), and from the Illinois Natural History Survey, Champaign, IL, USA (INHS), respectively. Although De Santis (1981) included males among the paratypes, none was found at UNLP. Similarly, for *E. versicolor*, males were reported as part of the type series (Girault, 1908), but could not be located in any of the museums indicated as depositories of types and cotypes by Girault. Slide-mounted individuals (four females and five males) of the dark form collected from *Trialeurodes packardi*

Table 1. Specimens used for molecular analyses.

Code	Locality	Date	Host	Revised species name
EPCA1–3	Portici, Italy (laboratory culture)	v.2014	*Trialeurodes vaporariorum*	*Encarsia gennaroi*
EPCAW1–5	Berkeley, CA, USA	vii.2015	*Trialeurodes* sp.	*Encarsia gennaroi*
EPTXW1–4	Weslaco, TX, USA	iii.2003	*Bemisia tabaci*	*Encarsia gennaroi*
EPIT1–2	Portici, Italy	v.2004	*T. vaporariorum*	*Encarsia gennaroi*
EPCI1–5	San Nicolás, Canary Islands, Spain	xii.2002	*Trialeurodes* sp.	*Encarsia gennaroi*
ETBR1–5	Sete Lagoas, Brazil (laboratory culture)	v.2014	*B. tabaci*	*Encarsia tabacivora*
ETTX1–5	Weslaco, TX, USA (laboratory culture)	x.2014	*B. tabaci*	*Encarsia suzannae*
ETTXW1–4	Weslaco, TX, USA	vii.2015	*B. tabaci*	*Encarsia suzannae*
ETTXW5	Weslaco, TX, USA	iii.2003	*B. tabaci*	*Encarsia suzannae*
E. sp. nr. *basicincta*	Kampala, Uganda	viii.2006	*Aleurothrixus floccosus*	–
E. citrina	Portici, Italy	ix.2002	*Parlatoria pergandei*	–

(Morrill) on *Impatiens pallida* Nutt. (Balsaminaceae) in Ithaca, NY, USA, and deposited at the Entomology Research Museum of the University of California, Riverside, USA (UCR), were also included in the morphometric analyses, along with three dark form females from Italy reared in the field from *T. vaporariorum*, hosted at the Museum of Entomology 'F. Silvestri', University of Naples 'Federico II', Portici, Italy (MES) (Fig. 1).

Species testing

The initial species hypothesis (H_0) we tested (illustrated in Fig. 1) was based on integration of all available information, including morphological, biological and geographical evidence. We hypothesized that: (i) the synonymy of *E. versicolor* with *E. pergandiella* was valid, not having *a priori* reasons to question it; (ii) the Texas light form was conspecific with *E. pergandiella* based on body colour, the presence of males and its North American distribution; (iii) the Brazil light form was conspecific with *E. tabacivora*, based on body colour, absence of males and known natural distribution confined to Brazil; and (iv) the dark form, including the laboratory culture and specimens from California, Texas, New York, Italy and the Canary Islands, represented a new species distinct from *E. pergandiella* and *E. tabacivora* based on body colour and sexual reproduction (relative to *E. tabacivora*). As molecular grade specimens were not available for type material of *E. pergandiella*, *E. tabacivora*, *E. versicolor* and for New York specimens, we tested H_0 by integrating the results of molecular and morphometric analyses to produce a final species hypothesis (H_1) (Fig. 1).

Molecular analyses

Genetic analyses were performed on samples listed in Table 1. DNA was extracted using a Chelex-proteinase K protocol as in Gebiola *et al.* (2009). A fragment of the mitochondrial gene cytochrome oxidase c subunit I (COI) and the D2 expansion region of the 28S rDNA gene (28S-D2) were sequenced. COI was amplified using the forward primers C1-J-2183 or C1-J-2195 with the reverse primer TL2-N-3014 (Simon *et al.,* 1994). 28S-D2 was amplified with primers D2F and D2R (Campbell *et al.,* 1993). PCR conditions for COI and 28S-D2

amplifications were as described in Gebiola *et al.* (2009). PCR products were cleaned and sequenced in both directions at the University of Arizona Genetics Core. Chromatograms were edited using BIOEDIT 7.0 (Hall, 1999). COI sequences were verified for protein coding frameshifts and nonsense codons using EMBOSS TRANSEQ (http://www.ebi.ac.uk/Tools/st/emboss_transeq/). Sequences were deposited in GenBank with accessions KT748657–KT748728.

The COI and 28S-D2 sequences were aligned using BIOEDIT and the online version of MAFFT 6 (G-INS-I algorithm) (Katoh & Toh, 2008), respectively. Phylogenies were obtained using maximum likelihood (ML) in RAxML 7.0.4 (Stamatakis, 2006) and bayesian inference (BI) in MRBAYES 3.2 (Ronquist *et al.,* 2012) on a supermatrix partitioned as 28S-D2, COI codon positions 1 and 2, COI codon position 3, and implementing the GTR + G evolutionary model, according to the best partitioning scheme found by PARTITIONFINDER (Lanfear *et al.,* 2012). ML trees were obtained after 1000 multiple inferences on the original alignment, starting from a random parsimonious tree and default initial rearrangement settings and number of rate categories. ML branch support was based on 1000 rapid bootstrap pseudoreplicates, and clades were considered as supported when bootstrap values were > 70%. For BI, two parallel runs of four simultaneous Monte Carlo Markov chains were run for one million generations, and trees were sampled every 1000 generations. Convergence of the separate runs was checked using the average deviation of split frequencies diagnostic (< 0.01) and the potential scale reduction factor (close to 1.00 for all parameters). The burn-in value was set at 25% of sampled topologies, and post-burn-in trees were summarized as a 50% majority rule consensus tree with posterior probabilities as nodal support and the threshold for clade acceptance set at 0.95. 28S-D2 sequences of *E. pergandiella* from Australia and California available in GenBank were added to the ingroup. *Encarsia citrina* (Crawford) and *Encarsia* sp. near *basicincta* Gahan, here sequenced as described earlier, were used as outgroups along with all other *Encarsia* species for which both 28S and COI sequences are available in GenBank. Uncorrected intra- and interspecific *p*-distances based on COI were calculated by MEGA6 (Tamura *et al.,* 2013).
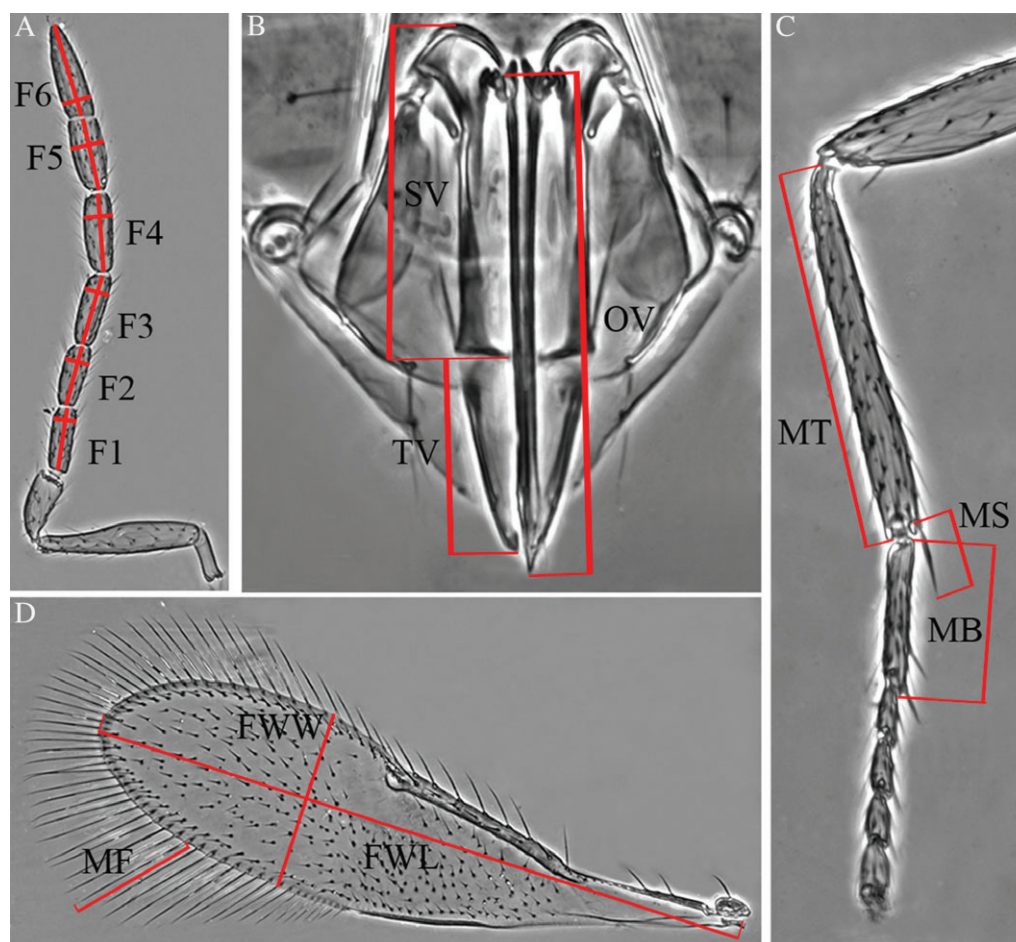
Fig. 2. Schematic representation of measurements taken. (A) Antenna; (B) ovipositor; (C) midleg; (D) forewing. FWL, length of forewing; FWW, maximum width of forewing; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus; MS, length of midspur; OV, length of ovipositor; SV, length of second valvula; and TV, length of third valvula as maximum internal length. [Colour figure can be viewed at wileyonlinelibrary.com].

Morphometric analyses

Specimens from laboratory cultures or collected in the field were mounted on slides in Canada Balsam, according to Noyes (1982). For the laboratory cultures, we mounted 15 females reared from *T. vaporariorum* and 8 from *B. tabaci* for the Brazil light form, 14 females reared from *T. vaporariorum* and 5 from *B. tabaci* for the Texas light form, and 14 females reared from *T. vaporariorum* and 6 from *B. tabaci* for the dark form. In addition, individuals of the dark form from Canary Islands (2 females) and from Berkeley, California (2 females), were also mounted. We also included in the analysis 25 male specimens from laboratory cultures: 8 males for both the dark form and Texas light form reared on *E. formosa* (itself reared on *B. tabaci*), plus 9 males for the dark form reared on *E. formosa* (itself reared on *T. vaporariorum*). Not all individuals of the type series were used for morphometric analyses because the poor slide-mounting of many specimens made observation of several morphological characters impossible. Consequently, the *E. pergandiella*

'holotype' (see remarks later) was discarded because the ovipositor was not visible, while for *E. tabacivora* and *E. versicolor*, four paratypes and three syntypes were discarded, respectively. Antenna of specimens of the latter two species were deformed (collapsed or squashed), and therefore the width of flagellar segments, even if measured, was not considered informative. In total, 98 females and 30 males belonging to museum collections or newly slide-mounted (from both field and laboratory material) were selected for morphometric analyses.

Measurements were taken with a Zeiss Axiophot 2 phase contrast microscope (Carl Zeiss, Oberkochen, Germany) at 200× and 400× magnification. The following continuous characters were measured: F1L–F6L, length of flagellar segments of antenna; F1W–F6W, maximum width of flagellar segments of antenna; FWL, length of forewing, measured as the distance between its most apical point and its base underneath the tegula; FWW, maximum width of forewing, measured perpendicular to FWL; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus;

MS, length of midspur. In addition, for females we also measured the following: OV, length of ovipositor, measured as the distance between the proximal margin of the basal ring to the extreme apex; SV, length of second valvula; and TV, length of third valvula as maximum internal length. Measurements of continuous characters are illustrated in Fig. 2. In the case of paired structures, each character was expressed as the mean of the two measures. As it was not possible to measure the SV and TV on specimens of *E. versicolor*, two datasets were analyzed: the first including *E. versicolor* but excluding the measurements of SV and TV, and a second dataset excluding *E. versicolor* and including SV and TV. Among the discrete characters, we considered the numbers of setae on the sixth tergites, reported by Myartseva & Evans (2008) as discriminant between their concepts of *E. pergandiella* (two pairs of setae on the sixth tergite) and *E. tabacivora* (one pair of setae on the sixth tergite). Slide-mounted material used for morphometric analysis will be deposited partly at the Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, SS di Portici, Napoli, Italy (IPSP) and partly at USNM.

Preliminary univariate analysis of raw morphological data revealed the existence of overall size differences among populations, which was in part host-induced (see the Results). Variables were therefore used to calculate specific ratios traditionally employed in the taxonomy of *Encarsia*, which were subjected to univariate analysis.

To factor out size as a nuisance variable in multivariate analyses, we implemented two different approaches. In the first approach, we used the size correction method developed by Bookstein and colleagues (Humphries *et al.,* 1981; Bookstein *et al.,* 1985; Rohlf & Bookstein, 1987), which uses Burnaby's size correction method (Burnaby principal component analysis, BPCA) (Burnaby, 1966), implemented by the program BURN-ABY PCA (available from N. MacLeod, Natural History Museum, London; http://www.nhm.ac.uk/hosted_sites/paleonet/ftp/ftp.html). Rohlf & Bookstein (1987) considered treating the original morphometric data by BPCA for size adjustment to be appropriate when all character coefficients of the first principal component (PC1) of the variance-covariance matrix have the same sign and similar magnitude. In this analysis, PC1 is assumed to be a size vector (Jolicoeur, 1963; Bookstein *et al.,* 1985), while the remaining PCs represent shape effects. The size effect is then removed from the data matrix by projecting the log-transformed data onto a plane orthogonal to that defined by PC1. This method produces, in addition to the aforementioned PCs, size-free 'raw' variables ('backprojected data'), which can be used in standard multivariate analyses (MVAs). Prior to BPCA, all morphometric characters were standardized, dividing each variable by its overall geometric mean, to have all measures in the same order of magnitude, thus avoiding dominance of larger characters over the others (e.g. FWL versus MS) (Claude, 2008). The backprojected data were subjected to a canonical principal component analysis (PCA) in order to discover the existence of groupings without any *a priori* assignment of specimens to a particular species. These results were then used in a linear discriminant analysis (LDA) to evaluate

if the morphometric variables could discriminate among specimens using the groups found in the PCA as class variables. The stability of the discriminant functions was verified by jackknife resampling, excluding one specimen at a time and identifying it by the new discriminant function. All statistical analyses were performed with STATGRAPHICS (1997).

The second approach we used is based on the conversion of original characters into ratios, advocated by Mosimann and colleagues (Mosimann, 1970, 1988; Darroch & Mosimann, 1985; Jungers *et al.,* 1995). Ratios are intuitive, represent descriptors of body proportions, and have been traditionally employed in species descriptions of many animal groups (Mayr & Ashlock, 1991), including parasitoid wasps (Noyes, 2004; Horstmann, 2009). Their use in multivariate analysis has been criticized, due to some statistical limitations (e.g. a potential increase in spurious correlations in the covariance matrix; Atchley *et al.,* 1976, but see reply of Hills, 1978). Multivariate ratio analysis (MRA), originally developed by Baur & Leuenberger (2011), has elegantly addressed such limitations in the use of ratios, besides proving very effective in identifying new diagnostic ratios for different groups and allowing an immediate inference on allometry (Baur *et al.,* 2014). This method works in multivariate ratio space but does not require establishing any predefined ratios. Moreover, allometry is not a prerequisite for the analysis but is treated as a hypothesis to be tested after the size values are determined, and size and shape are independent. Using the same conceptual framework for size and shape, MRA allows interpretation of PCA and LDA in terms of body ratios, thus fully exploiting the insights gained by MVA for species description and diagnosis. MRA consists of different tools: shape PCA, PCA ratio spectrum, allometry ratio spectrum and LDA ratio extractor. The shape PCA, after definition of an isometric size axis (isosize), calculated as the geometric mean of all variables, is a PCA performed in the space of all ratios to evaluate the existence of hidden multivariate structure in the samples. The most important shape PCs are then plotted against the isosize to visually inspect the correlation of shape with size to estimate data allometry. The PCA ratio spectrum is a graphical tool that attempts to explain shape PCs in terms of body ratios. The LDA ratio extractor finds the ratios that best discriminate the groups previously identified by shape PCA. These ratios do not necessarily coincide with ratios traditionally used in the taxonomy of the group under study. These ratios are then evaluated with another graphical tool, the allometry ratio spectrum, which is used to check allometric behaviour of characters used for the calculation of diagnostic ratios. The R language and environment for statistical computing was used for data analysis (R Core Team, 2015). In particular, we employed slightly modified versions of the R-scripts provided by Baur *et al.* (2014).

Results

Molecular analyses

Trimmed COI sequences resulted in a 757-bp fragment. Alignment was straightforward with no frame shifts, nonsense
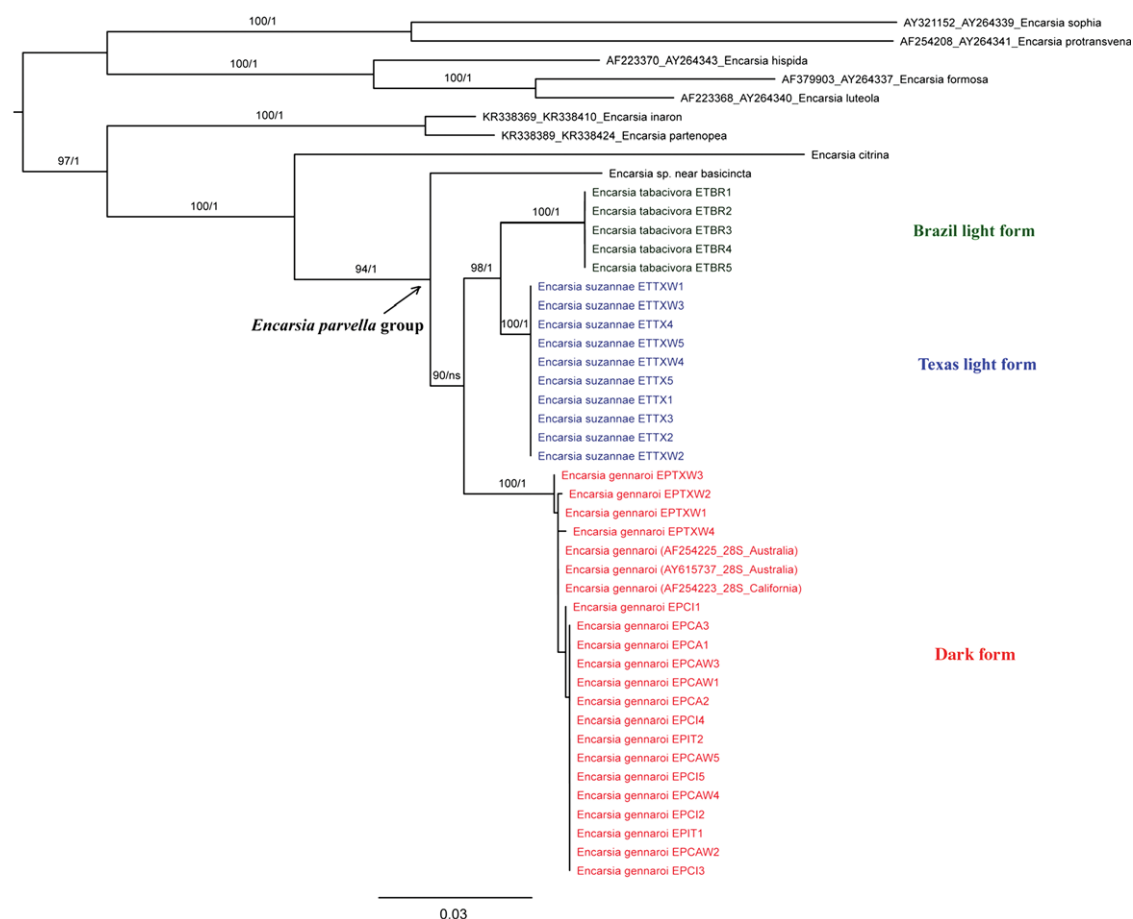
Fig. 3. Maximum likelihood tree based on a concatenated 28S-D2 and cytochrome *c* oxidase subunit I dataset. Bootstrap values ≥70 and posterior probabilities ≥0.95 for a topologically identical majority rule Bayesian consensus tree are shown above branches. [Colour figure can be viewed at wileyonlinelibrary.com].

codons, insertions or deletions identified in any sequence. Trimmed 28S-D2 sequences for the three focal taxa were 688 bp, and the final alignment consisted of 702 bp. ML and BI analyses based on the concatenated dataset resulted in trees of identical topology, and supported the distinction between two main groups: one including only individuals of the dark form (laboratory culture, field specimens from California, Texas, Italy and the Canary Islands), the other including only individuals of the light form, with two reciprocally monophyletic groups: the Texas light form (laboratory culture and field specimens) characterized by sexual reproduction, and the Brazil light form (laboratory culture) characterized by thelytokous reproduction (Fig. 3). These two light forms have identical 28S-D2 sequences, but clear differences in COI sequences. As for the dark form, all individuals examined showed no difference in 28S-D2, and were identical to 28S-D2 sequences of individuals from Australia and California retrieved from GenBank as *E. pergandiella*. 28S-D2 sequences were different between the dark form and the Texas and Brazil light forms. A single COI haplotype was recovered for each of the light forms. Sequence variation was, however, recorded within the dark form, with a common haplotype shared by the laboratory culture and field-collected individuals

from California, the Canary Islands and Italy, but which is not present in Texas, where the highest variation was recorded (four haplotypes out of four sequenced specimens), suggesting that this could be the area of origin of the dark form. There were 34 synonymous and four nonsynonymous substitutions between the dark form and the Brazil light form, 24 and one between the dark form and the Texas light form, and 24 and three between the Brazil light form and the Texas light form, respectively. The average uncorrected COI *p*-distances between the dark form and the Brazil and Texas light forms were 5.0 and 3.7%, respectively, while the sister Brazil and Texas light forms differed by 3.4% (Table S1). In summary, molecular analyses showed that the light and dark forms are not the result of intraspecific variation, indicating species status for the dark form, the Brazil light form and Texas light form.

Morphometric analyses

Univariate analysis.
The original data did not permit meaningful comparisons due to large differences in the average size of the seven studied groups,

Table 2. Mean ratios (standard deviation) over variation range of selected ratios of morphological characters of *Encarsia pergandiella* species complex females.

Ratios	*E. pergandiella* (*n* = 5)	*Encarsia versicolor* (*n* = 8*)	*Encarsia marthae* (New York dark form) (*n* = 4)	*Encarsia tabacivora* types (*n* = 12)	*Encarsia tabacivora* (Brazil light form) (*n* = 23)	*Encarsia suzannae* (Texas light form) (*n* = 19)	*Encarsia gennaroi* (dark form) (*n* = 27)
F1L/F2L	0.88 (0.022)[c]	0.87 (0.046)[c]	0.89 (0.013)[c]	0.81 (0.048)[ab]	0.81 (0.032)[a]	0.85 (0.048)[bc]	0.98 (0.060)[d]
	0.85–0.90	0.81–0.94	0.87–0.90	0.76–0.90	0.71–0.87	0.74–0.91	0.85–1.11
F1L/F3L	0.74 (0.058)[a]	0.72 (0.037)[a]	0.74 (0.049)[a]	0.71 (0.046)[a]	0.71 (0.030)[a]	0.73 (0.037)[a]	0.86 (0.059)[b]
	0.67–0.80	0.66–0.78	0.68–0.78	0.63–0.78	0.61–0.77	0.65–0.78	0.75–0.98
F2L/F3L	0.84 (0.052)[abc]	0.82 (0.053)[a]	0.83 (0.043)[ab]	0.87 (0.035)[cd]	0.87 (0.021)[cd]	0.86 (0.032)[bc]	0.88 (0.026)[e]
	0.79–0.89	0.76–0.93	0.78–0.87	0.81–0.92	0.83–0.90	0.81–0.92	0.83–0.94
F3L/F4L	0.93 (0.029)[a]	0.94 (0.032)[a]	0.99 (0.085)[b]	0.95 (0.039)[a]	0.94 (0.027)[a]	0.93 (0.041)[a]	0.92 (0.036)[a]
	0.90–0.97	0.87–0.97	0.93–1.12	0.87–1.00	0.89–1.00	0.88–1.01	0.83–0.97
F4L/F5L	1.02 (0.047)[c]	1.03 (0.047)[c]	0.99 (0.050)[bc]	0.90 (0.027)[a]	0.93 (0.018)[a]	0.96 (0.041)[b]	0.98 (0.042)[b]
	0.96–1.07	0.98–1.11	0.94–1.05	0.85–0.93	0.89–0.95	0.88–1.02	0.87–1.06
F5L/F6L	0.85 (0.009)[bc]	0.86 (0.026)[c]	0.86 (0.041)[c]	0.81 (0.035)[ab]	0.82 (0.022)[ab]	0.80 (0.033)[a]	0.81 (0.043)[a]
	0.84–0.86	0.82–0.90	0.82–0.91	0.76–0.89	0.76–0.85	0.74–0.85	0.72–0.90
F1L/F1W	2.29 (0.195)[b]	–	2.33 (0.046)[bc]	–	2.09 (0.153)[ab]	1.99 (0.205)[a]	2.55 (0.322)[c]
	2.00–2.43		2.29–2.40		1.80–2.32	1.67–2.33	1.81–3.04
FWL/FWW	3.56 (0.155)[abc]	3.70 (0.278)[c]	3.54 (0.026)[abc]	3.57 (0.103)[ab]	3.65 (0.135)[bc]	3.50 (0.090)[a]	3.61 (0.121)[bc]
	3.41–3.74	3.19–4.08	3.52–3.57	3.45–3.75	3.40–3.94	3.32–3.65	3.33–3.84
MF/FWW	0.78 (0.040)[cd]	0.77 (0.048)[cd]	0.67 (0.011)[a]	0.81 (0.054)[d]	0.77 (0.065)[c]	0.75 (0.044)[bc]	0.72 (0.045)[ab]
	0.74–0.83	0.71–0.84	0.65–0.68	0.71–0.91	0.67–0.89	0.68–0.84	0.63–0.81
OV/MT	1.00 (0.013)[e]	1.01 (0.052)[e]	0.88 (0.020)[cd]	0.82 (0.031)[b]	0.76 (0.029)[a]	0.89 (0.050)[d]	0.85 (0.063)[bc]
	0.99–1.02	0.94–1.07	0.86–0.90	0.75–0.87	0.70–0.82	0.79–0.98	0.74–1.04
OV/SV	1.76 (0.098)[d]	–	1.33 (0.034)[a]	1.49 (0.076)[bc]	1.45 (0.070)[b]	1.51 (0.105)[c]	1.47 (0.058)[bc]
	1.69–1.90		1.30–1.38	1.38–1.66	1.34–1.58	1.30–1.64	1.37–1.62
OV/TV	2.48 (0.277)[ab]	–	2.30 (0.207)[a]	2.56 (0.138)[b]	2.53 (0.123)[b]	2.51 (0.166)[b]	2.51 (0.115)[b]
	2.24–2.82		2.10–2.59	2.28–2.73	2.33–2.75	2.20–2.80	2.30–2.75
OV/MB	2.48 (0.132)[d]	2.35 (0.116)[cd]	1.96 (0.049)[ab]	1.95 (0.120)[b]	1.85 (0.087)[a]	2.26 (0.110)[c]	1.87 (0.163)[ab]
	2.35–2.67	2.14–2.46	1.89–1.99	1.77–2.18	1.70–2.03	2.07–2.41	1.58–2.37
SV/TV	1.41 (0.096)[a]	–	1.74 (0.174)[bc]	1.72 (0.096)[bc]	1.75 (0.090)[c]	1.67 (0.071)[b]	1.71 (0.079)[bc]
	1.31–1.51		1.53–1.95	1.59–1.94	1.60–1.95	1.56–1.81	1.55–1.90
MT/MB	2.48 (0.102)[de]	2.31 (0.135)[bc]	2.23 (0.0431)[ab]	2.39 (0.103)[cd]	2.43 (0.065)[d]	2.56 (0.117)[e]	2.21 (0.050)[a]
	2.37–2.61	2.15–2.58	2.20–2.29	2.21–2.62	2.33–2.61	2.37–2.81	2.11–2.31
MB/MS	2.40 (0.126)[bc]	2.52 (0.205)[cd]	2.78 (0.125)[e]	2.38 (0.153)[bc]	2.29 (0.121)[b]	1.96 (0.103)[a]	2.60 (0.230)[de]
	2.24–2.54	2.33–2.99	2.62–2.92	2.20–2.75	2.06–2.48	1.69–2.15	2.07–3.09

F1L–F6L, length of flagellar segments of antenna; F1W–F6W, maximum width of flagellar segments of antenna; FWL, length of forewing; FWW, maximum width of forewing; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus; MS, length of midspur; OV, length of ovipositor; SV, length of second valvula; and TV, length of third valvula as maximum internal length. **n* = 6 for MF/FWW and OV/MT. Different letters indicate significant differences (95%) by multiple range test (Tukey's honestly significant difference).

as pointed out by MT, chosen as a size indicator (Table S2). In particular, females of the dark form (including New York) together with *E. pergandiella* types had an average longest MT, while females of the Texas light form and *E. tabacivora* types had the shortest. These differences may be in part host-induced, but may also reflect true species-specific morphological differences. Indeed, comparisons among the three laboratory cultures revealed that parasitoids emerging from *B. tabaci* are smaller than those reared from *T. vaporariorum* within each culture, but females of the dark form tend to be larger than those of the other two, even when reared from the same whitefly host species (Table S3). The whitefly host effect on parasitoid size was also evident from analysis of male measurements, with individuals of the dark form reared from *E. formosa* on the whitefly

T. vaporariorum having MTs that were significantly longer than those reared from the same wasp on *B. tabaci* (Table S4).

Univariate analyses on ratios from female data (Table 2) showed that, although many ratios overlapped, a few of them, if used in combination, were able to separate some groups from the others. For example, *E. pergandiella* and *E. versicolor* had the highest OV/MT values; *E. pergandiella* had the highest OV/SV and the lowest SV/TV values, the dark form had the highest F1L/F3L value, while F4L/F5L could discriminate *E. pergandiella* from *E. tabacivora* types and the Brazil light form. Partitioning ratios by laboratory hosts revealed differences both within and between populations In particular, one (MF/FWW), four (F4L/F5L, OV/SV, OV/TV, MT/MB) and three (F1L/F3L, OV/MT, MB/MS) ratios for the Brazil light

Table 3. Mean ratios (standard deviation) over variation range of selected ratios of morphological characters of *Encarsia pergandiella* species complex males.

Ratios	*Encarsia marthae* (New York dark form) (n = 5)	*Encarsia gennaroi* (dark form) n = 17	*Encarsia suzannae* (Texas light form) n = 8
F1W/F2W	1.12 (0.056)[a] 1.04–1.19	1.04 (0.063)[a] 0.96–1.17	1.09 (0.059)[a] 1.02–1.19
F1L/F1W	1.71 (0.150)[a] 1.50–1.89	2.48 (0.242)[b] 2.13–2.97	1.87 (0.189)[a] 1.51–2.10
F2L/F2W	2.18 (0.238)[a] 1.89–2.47	2.64 (0.174)[b] 2.41–2.93	2.12 (0.185)[a] 1.83–2.31
F1L/F2L	0.87 (0.027)[a] 0.84–0.91	0.98 (0.055)[b] 0.89–1.08	0.96 (0.037)[b] 0.90–1.02
F1L/F3L	0.87 (0.016)[b] 0.86–0.89	0.98 (0.050)[a] 0.89–1.05	0.92 (0.042)[b] 0.87–0.98
F2L/F3L	0.99 (0.019)[ab] 0.97–1.02	1.00 (0.031)[a] 0.97–1.07	0.96 (0.051)[b] 0.87–1.01
F3L/F4L	0.99 (0.013)[a] 0.98–1.01	0.97 (0.041)[a] 0.88–1.05	0.99 (0.039)[a] 0.94–1.05
F4L/F5L	1.05 (0.006)[ab] 1.04–1.06	1.06 (0.051)[a] 0.94–1.18	1.01 (0.022)[b] 0.98–1.05
F5L/F6L	0.94 (0.018)[a] 0.92–0.96	0.90 (0.064)[a] 0.76–1.00	0.89 (0.038)[a] 0.84–0.95
FWL/FWW	3.41 (0.129)[b] 3.30–3.62	3.40 (0.114)[b] 3.29–3.77	3.18 (0.092)[a] 3.01–3.28
MF/FWW	0.67 (0.030)[a] 0.61–0.70	0.69 (0.047)[a] 0.62–0.80	0.70 (0.043)[a] 0.65–0.75
MT/MB	2.50 (0.115)[b] 2.36–2.63	2.36 (0.067)[a] 2.23–2.45	2.79 (0.112)[c] 2.71–3.02
MB/MS	2.19 (0.248)[b] 1.83–2.51	2.19 (0.131)[b] 2.03–2.50	1.73 (0.111)[a] 1.60–1.88

F1L–F6L, length of flagellar segments of antenna; F1W–F6W, maximum width of flagellar segments of antenna; FWL, length of forewing; FWW, maximum width of forewing; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus; MS, length of midspur. Different letters indicates significant differences (95%) by multiple range test (Tukey's HSD).

form, the Texas light form and dark form, respectively, showed host-associated intraspecific differences (Table S5). The most evident differences found in the ratios from male data (Table 3) are due to the highest F1L/F1W value for the California population and the highest MT/MB and the lowest MB/MS values for the Texas population. The differences highlighted using this method may be rather subtle and the range of variation may sometimes overlap, making it difficult to distinguish true differences from artifacts, which can occur during slide mounting. Regarding the number of setae on the sixth tergite, we found invariably only one pair in all the observed wasps (with the exclusion of one specimen of *E. pergandiella* possessing three setae), except for the New York dark form, which showed two pairs of setae for both sexes.

Principal component analysis

In a preliminary run of BPCA, coefficients of PC1 had the same sign and comparable values for all characters, with the exception of MF (which had a positive sign but was one order of magnitude less than other ones for females, and had a negative sign for males). This character was therefore excluded in the final analysis. After MF removal, the assumption of uniform character coefficients was satisfied and PC1 was considered an effective size vector, and the resulting backprojected data as size-free. The PCA run on the first set of backprojected data (SV and TV excluded) of female specimens was able to separate five groups: *E. pergandiella* + *E. versicolor*; the dark form; the New York dark form; the Texas light form; and *E. tabacivora* + the Brazil light form (data not shown). On this basis and considering their very similar OV/MT ratio and body colour, the synonymy of *E. versicolor* and *E. pergandiella*, previously proposed by other authors, was regarded as valid. Consequently, and also because of the poor quality of the type material, *E. versicolor* specimens were not included in subsequent analyses. The PCA analysis run on the second set of backprojected data (SV and TV included) was simplified excluding FWL and FWW. All characters (except MS) provided substantial contributions to either PC (Table S6). Three nonoverlapping groups were recovered on the first two PC axes: *E. tabacivora* + the Brazil light form, the dark form, and the Texas light form partially overlapping with both *E. pergandiella* and the New York dark form (Fig. 4A). However, these latter three populations were clearly separated in several other contrasts among PCs (data not shown). By contrast, *E. tabacivora* and the Brazil light form could not be completely separated in any of the other PC combinations. The same analysis performed on the male specimens recovered three separate groups (the dark form, the New York dark form and the Texas light form) (Fig. 4C). The PCA on the backprojected data extracted four significant components, with all characters (except F6L) contributing with similar weight to PCs (Table S6).

Linear discriminant analysis

Clusters found by PCA were used as classification factors for a LDA performed on backprojected data for both sexes. Thus, *E. tabacivora* and the Brazil light form were considered as a single group. The LDA on the backprojected data of female specimens found four significant linear discriminant functions (LDs). When female specimens were projected into the space defined by the first two LDs, three groups (the dark form, the Texas light form, and *E. tabacivora* + the Brazil light form) were clearly discriminated, whereas *E. pergandiella* and the New York dark form partially overlapped with the Texas light form and the dark form, respectively (Fig. 4B). However, the four LDs taken together clearly separated five groups and all specimens were correctly assigned to the corresponding *a priori* group. A stability test confirmed the efficacy of the LDA, with 97.8% of individually excluded specimens correctly classified. Based on the relative magnitude of the discriminant function coefficients, all characters were important contributors for discriminating among groups (Table S7). The relationship between *E. tabacivora* and the Brazil light form was further assessed by considering them as separate groups. In this analysis, the centroids of the two groups were closely located in the LD1 and
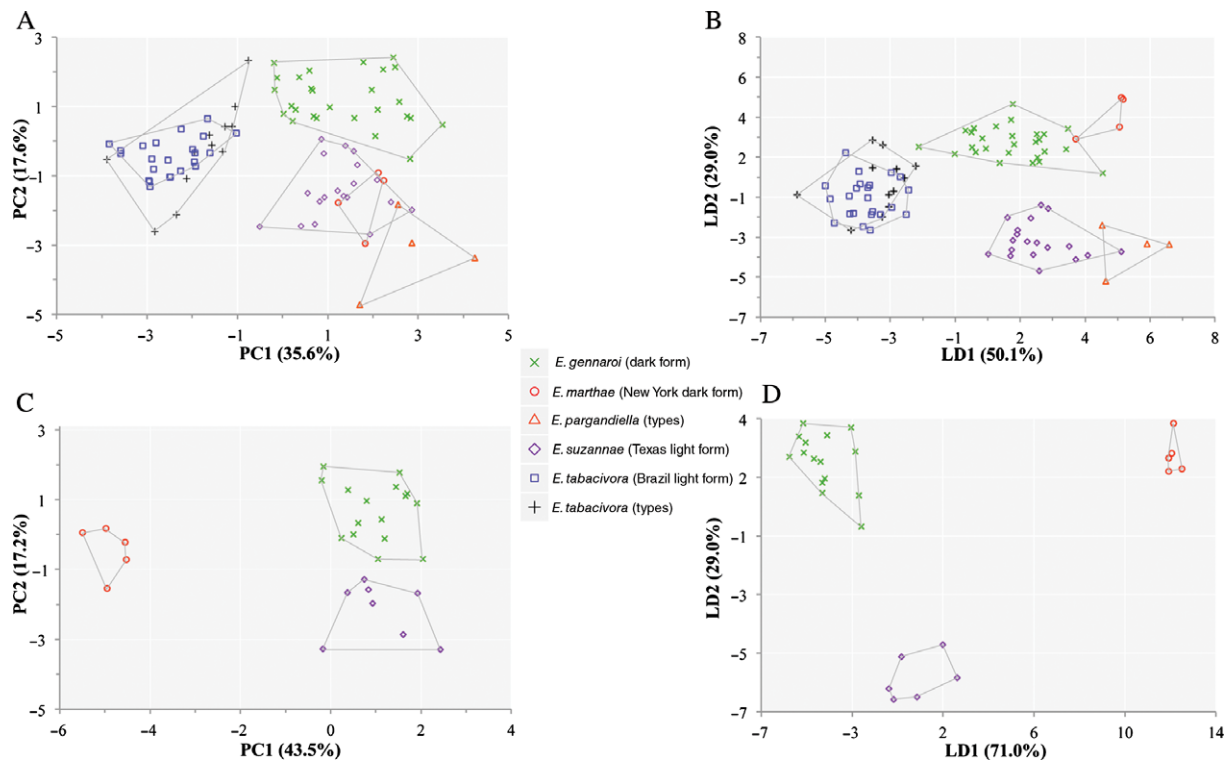
Fig. 4. Scatterplots of principal component analysis (PCA) and linear discriminant analysis (LDA). First two principal components (PC1 and PC2) and linear discriminant functions (LD1 and LD2) using backprojected data. (A) PCA on females; (B) LDA on females; (C) PCA on males; (D) LDA on males. [Colour figure can be viewed at wileyonlinelibrary.com].

LD2 space, and several specimens (16.7% for *E. tabacivora* and 8.7% for the Brazil light form) were incorrectly classified, indicating a large overlap between these groups (data not shown), thus supporting the PCA results, i.e. the conspecificity of the two groups.

Similar results were obtained for the male specimens projected on the space defined by the two LDs, which clustered in three completely distinct groups (Fig. 4D). All specimens were correctly classified, with all characters contributing with comparable coefficients to both LDs, and the F1L coefficient expressing the highest values (Table S7).

Multivariate ratio analysis

The shape PCA from MRA was largely congruent with the PCA on backprojected data. Three components were extracted. On the first two PCs, four groups marginally overlapping were recovered: *E. tabacivora* + the Brazil light form, the dark form, the Texas light form, and *E. pergandiella* (Fig. 5A). Specimens of the New York dark form grouped within the dark form. In the other contrasts, all the groups, including the New York dark form, were identified as a separate group, with the exception of a slight overlapping between the dark form and the Texas light form (data not shown). Plotting the isosize against shape PC1 showed a fairly large amount of allometry in the dataset (Fig. 5B). By performing intraspecific shape PCA by species for which we have populations reared on two laboratory

hosts, *T. vaporariorum* and *B. tabaci*, we confirmed that the allometric behaviour is mostly due to specimens of the dark form (data not shown). Characters defining the ratios that mostly contributed to the first PC are shown in Fig. 5C. Moreover, the most important ratio for the first shape PC (i.e. defined by the most distant characters on the PCA ratio spectrum, i.e. F1L/MS) was also the most allometric (Fig. 5D). MRA on male specimens confirmed results of the PCA on backprojected data, i.e. the dark form, the New York dark form and the Texas light form were easily identified as separate groups by the first two shape PCs (Fig. 6A). The male dataset showed a reduced allometry compared with females (Fig. 6B). Characters defining the ratios that mostly contributed to the first PC are shown in Fig. 6C. The LDA ratio extractor identified several ratios for both females and males, mostly new to *Encarsia* taxonomy (Table 4), which we subsequently used for species diagnoses and for the identification key. Most diagnostic ratios were the least allometric ones for females, and moderately allometric for males, respectively (Figs 5D, 6D).

Discussion

The morphometric and molecular analyses conducted here showed that the *E. pergandiella* species complex exhibits considerable cryptic diversity. A complex that was initially thought to include only two species (*E. pergandiella* and *E. tabacivora*),
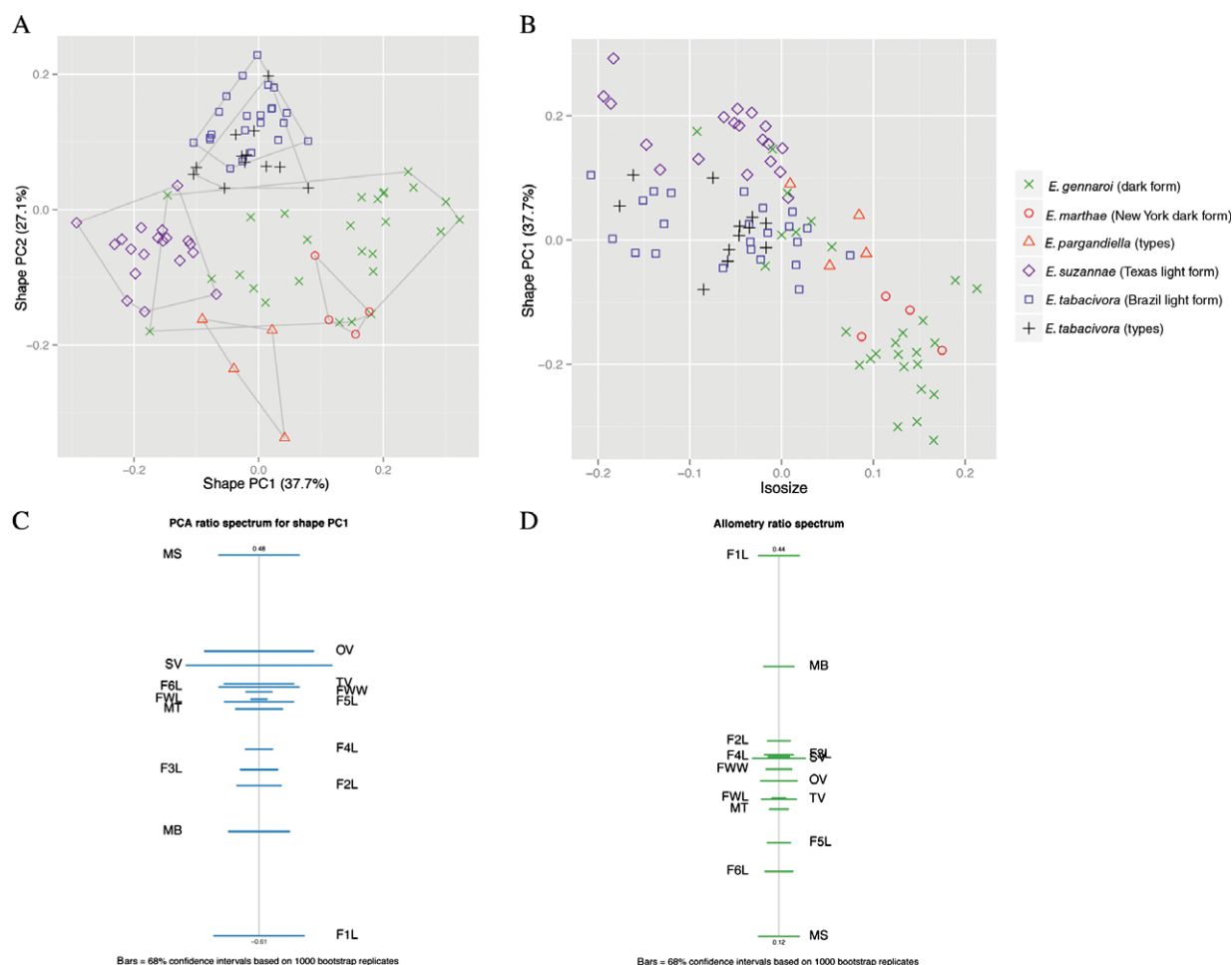
Fig. 5. Multivariate ratio analysis (MRA) on females. (A) Shape principal component analysis (PCA), scatterplot of first two shape components (PC1 and PC2); (B) scatterplot of isosize against the first shape PC; (C) PCA ratio spectrum; (D) allometry ratio spectrum. F1L–F6L, length of flagellar segments of antenna; F1W–F6W, maximum width of flagellar segments of antenna; FWL, length of forewing; FWW, maximum width of forewing; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus; MS, length of midspur; OV, length of ovipositor; SV, length of second valvula; and TV, length of third valvula as maximum internal length. [Colour figure can be viewed at wileyonlinelibrary.com].

and that in our initial hypothesis (H_0) would include only an additional third species, comprises in fact five species, three of them new to science (H_1). The three new species are formally described for the first time, and can be distinguished by a combination of molecular markers, morphological and biological traits. Phylogenetic analyses based on the concatenated COI and 28S-D2 sequences supported the distinction between the dark form and the Brazil and Texas light forms, according to the body colour and biological features. All sequenced individuals of the face-down pupating dark form, regardless of their geographic origin, formed a well supported clade. All individuals of the face-up pupating light form have identical 28S-D2 sequences, but group in two clades, Texas (biparental) and Brazil (uniparental), due to clear differences in COI sequences. Also within the dark form, all examined individuals showed no difference in the sequence of 28S-D2, whereas a large 28S-D2 sequence variation differentiated the dark form from the Texas and Brazil light

forms. This result is consistent with previous studies showing that the region 28S-D2 is conserved, if not invariant, among closely related species of chalcidoids that are well differentiated biologically and for the COI sequence (Heraty *et al.,* 2007; Gebiola *et al.,* 2010). As an example, a single 28S-D2 polymorphism has been found to discriminate cryptic species characterized by consistent variation in COI sequences in Eulophidae (Gebiola *et al.,* 2009; Nugnes *et al.,* 2015), as well as in the genus *Encarsia* (Gebiola *et al.*, 2016). As for COI, the level of divergence found between the three lineages (3.4–5%) is similar to or even higher than that found between cryptic species of other parasitoid wasps (Heraty *et al.,* 2007; Gebiola *et al.*, 2016; Nugnes *et al.,* 2015).

The species status of the dark form, Brazil light form and Texas light form was also supported by morphometric analysis. In this study, morphological characterization of species within the *E. pergandiella* complex was almost exclusively based
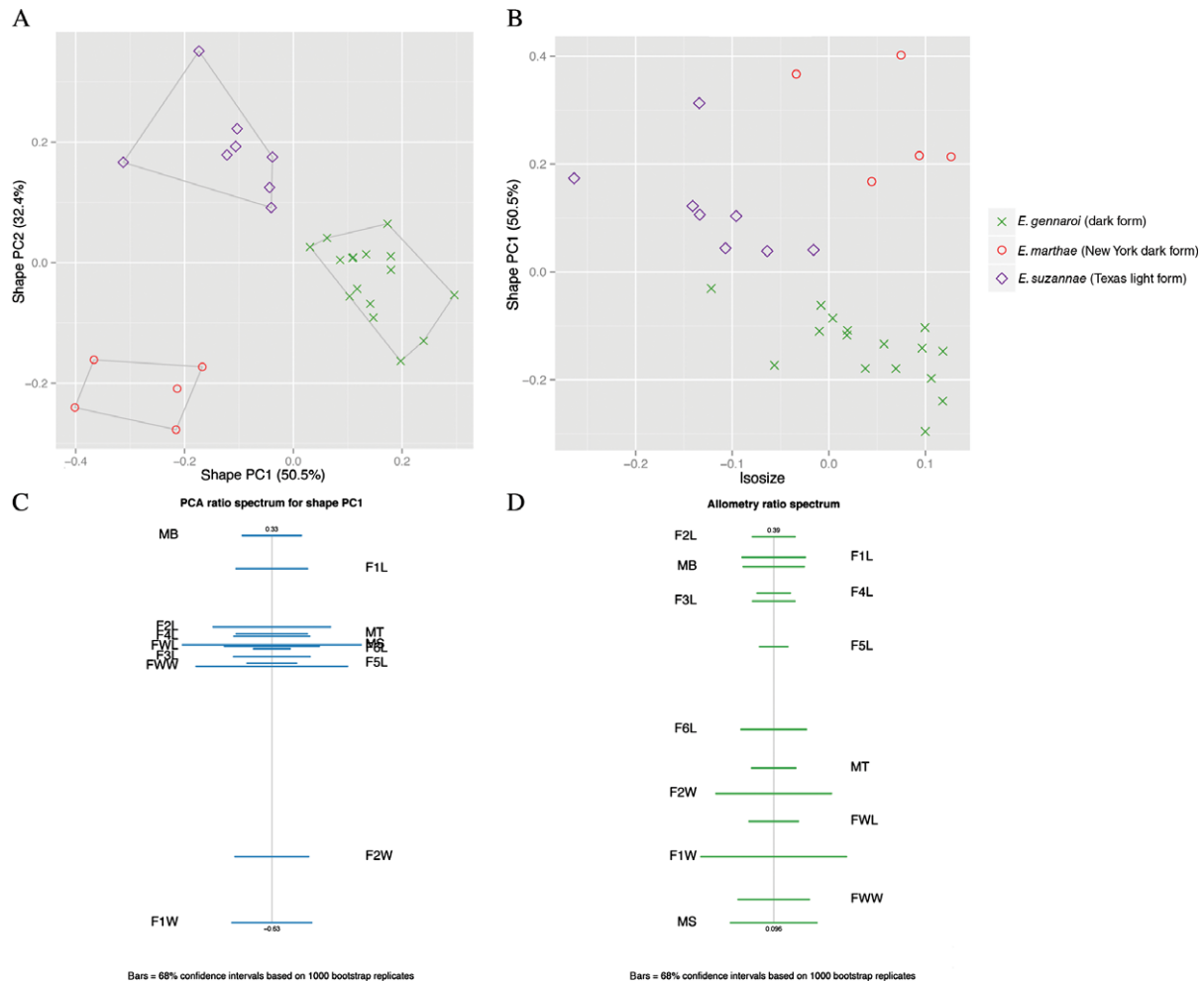
Fig. 6. Multivariate ratio analysis (MRA) on males. (A) Shape principal component analysis (PCA), scatterplot of first two shape components (PC1 and PC2); (B) scatterplot of isosize against the first shape PC; (C) PCA ratio spectrum; (D) allometry ratio spectrum. F1L–F6L, length of flagellar segments of antenna; F1W–F6W, maximum width of flagellar segments of antenna; FWL, length of forewing; FWW, maximum width of forewing; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus; MS, length of midspur. [Colour figure can be viewed at wileyonlinelibrary.com].

on the observation of continuous characters. Among discrete characters we considered only the number of setae between cerci on the sixth tergite. Previously, Myartseva & Evans (2008) reported the presence of two pairs of setae in their concept of *E. pergandiella* (New York material included) and one pair in *E. tabacivora*. Our results are only partially in agreement with their observation. We found two pairs of setae only in individuals of the New York population while all other individuals examined had one pair of setae. As for continuous morphological characters, we found that while the average size of many of them differed significantly among populations, their ranges largely overlapped. Moreover, very few morphometric ratios traditionally used in the systematics of the genus *Encarsia* were discriminatory at the species level. OV/MT was first used by Myartseva & Evans (2008) to effectively identify *E. pergandiella*, which in our analysis was the only species of the complex with the ovipositor always subequal to the midtibia.

A ratio of approximately one was found in *E. versicolor* type series, even though the condition of the slide-mounted material did not permit a clear measurement of this character on all specimens, supporting the established synonymy with *E. pergandiella*. Myartseva & Evans (2008) also identified specimens from New York as *E. pergandiella*, reporting a ratio OV/MT ≥ 1, to justify this determination. However, the New York individuals we analysed showed the ratio OV/MT to be < 1, and the range of this ratio was not overlapping with that of *E. pergandiella*. It should be noted that the smaller specimens of the dark form (reared in the laboratory from *B. tabaci*) may have a higher ratio OV/MT compared with the larger ones, reaching in some cases the typical values found for *E. pergandiella* specimens. However, it should be stressed that specimens of *E. pergandiella* and the dark form with similar OV/MT values are very different in size and, when comparison is made between specimens of more uniform size classes, the two groups are very

Table 4. Best and second-best diagnostic ratios found by linear discriminant analysis ratio extractor for separating various groupings of females and males.

Group comparison	Best ratios	Range group 1	Range group 2
Females			
Encarsia marthae – rest	F3L/MT	0.29–0.34	0.24–0.30
	SV/MT	0.62–0.69	0.49–0.65
Encarsia pergandiella – rest	SV/TV	1.31–1.51	1.53–1.95
	MS/OV	0.16–0.18	0.17–0.26
Encarsia suzannae – rest	MB/FWW	0.43–0.49	0.47–0.60
	F5L/MS	1.37–1.58	1.49–2.15
Encarsia gennaroi – *E. marthae*	F3L/MB	0.53–0.62	0.64–0.79
	MT/FWL	0.33–0.37	0.31–0.33
E. gennaroi – *E. pergandiella*	F3L/MB	0.53–0.62	0.65–0.74
	SV/OV	0.62–0.73	0.53–0.59
E. gennaroi – *E. suzannae*	MB/FWW	0.52–0.60	0.43–0.49
	SV/MS	2.92–3.82	2.64–3.27
E. gennaroi – *E. tabacivora*	F1L/F2L	0.85–1.11	0.71–0.90
	F5L/OV	0.30–0.41	0.37–0.43
E. marthae – *E. suzannae*	F4L/MS	1.85–2.06	1.26–1.56
	SV/FWW	0.72–0.77	0.64–0.78
E. marthae – *Encarsia tabacivora*	F6L/SV	0.54–0.58	0.65–0.77
	MT/FWW	1.11–1.16	1.18–1.36
E. suzannae – *E. pergandiella*	F4L/MS	1.26–1.56	1.74–1.85
	TV/FWW	0.37–0.47	0.45–0.55
E. suzannae – *E. tabacivora*	F5L/TV	0.76–0.89	0.92–1.11
	MB/FWW	0.43–0.49	0.49–0.60
E. tabacivora – *E. pergandiella*	F5L/OV	0.37–0.43	0.28–0.33
	TV/MB	0.67–0.81	0.9–1.1
Males			
E. gennaroi – rest	F1W/MB	0.26–0.35	0.37–0.52
	F5L/MB	0.68–0.81	0.81–0.99
E. marthae – rest	F1W/MS	0.86–1.21	0.58–0.84
	F3L/MT	0.35–0.39	0.28–0.35
E. suzannae – rest	MB/MT	0.33–0.37	0.38–0.45
	F4L/MT	0.29–0.32	0.33–0.39
E. gennaroi – *E. marthae*	F1W/FWW	0.86–1.21	0.58–0.84
	F3L/MB	0.74–0.80	0.88–1.03
E. gennaroi – *E. suzannae*	MB/MT	0.41–0.45	0.33–0.37
	FWL/MS	14.13–16.78	13.21–15.97

F1L–F6L, length of flagellar segments of antenna; F1W–F6W, maximum width of flagellar segments of antenna; FWL, length of forewing; FWW, maximum width of forewing; MF, length of longest seta of the forewing marginal fringe; MT, length of midtibia; MB, length of midbasitarsus; MS, length of midspur; OV, length of ovipositor; SV, length of second valvula; and TV, length of third valvula as maximum internal length. When ratios are overlapping between groups, the combination of best and second-best ratios is diagnostic (see also Figure S1).

clearly distinguished. Other characters traditionally considered useful for species identification in *Encarsia* are the relative proportions of antennal segments, yet here only few of them were diagnostic. On the other hand, the ratio extractor analysis provided many new ratios diagnostic for nearly all pairwise contrasts, allowing an easy separation by a dichotomous key. Finally, individuals of the dark form (including New York) showed a distinctive colour pattern in both sexes that is clearly distinct from those of Texas and Brazil light forms, which in turn seem quite similar to the colour observed in slide-mounted material of *E. pergandiella* and *E. tabacivora*. These colour and morphometric differences are in agreement with genetic data indicating species divergence. Therefore, body colour, previously thought to be too variable to be a useful character, is in fact a diagnostic trait.

The three species identified on the basis of molecular markers are also true biological species, as they are reproductively isolated. The thelytokous Brazil light form is isolated because gene flow is prevented in one direction by the absence of Brazilian males (Zchori-Fein *et al.,* 2001; Kenyon & Hunter, 2007), and in the other direction by prezygotic isolation with both the dark form and the Texas light form (S.E. Kelly, M.S. Hunter, M. Giorgini and P.A. Pedata, unpublished data). Furthermore, allopatric and sympatric individuals of the dark form and Texas light form produce mostly sterile progeny when crossed (Johnson, 1996; M. Gebiola, S.E. Kelly, M. Giorgini and M.S. Hunter, unpublished data). Finally, the three populations show different behaviour during pupal formation, with the dark form pupating face down inside the host, and the Texas and Brazil light forms pupating face up (Johnson, 1996; Liu & Stansly, 1996b; M. Giorgini and P.A. Pedata, unpublished data). The phylogenetic sister relationship between the Texas and Brazil light forms, predictable based on external morphology and pupal orientation similarity, is interesting as it suggests that the diversification of *Cardinium* symbiont may have driven isolation in these two entities. Indeed, each harbours a specific strain of *Cardinium*, one inducing cytoplasmic incompatibility in the Texas light form and the other one causing thelytokous parthenogenesis in the Brazil light form (Zchori-Fein *et al.,* 2004). Finally, the species status of the New York dark form is also supported by biological traits. Indeed, the New York dark form is unique in having males able to develop also as primary parasitoids (within the body of whitely nymphs) because of the occurrence of a paternal sex ratio distorting factor that turns fertilized diploid eggs into haploid eggs (Hunter *et al.,* 1993).

Despite the lack of strong discriminating characters, the integration of morphometric traits (ratios), genetic and biological evidence effectively resolved the taxonomy of this species complex. Morphometric analyses also provided a sensitive method of distinguishing species in cases where only type material or museum specimens were available. As a result, we were able to: (i) confirm the validity of *E. pergandiella* and *E. tabacivora*; (ii) confirm the conspecificity of *E. versicolor* with *E. pergandiella*; (iii) establish that the Brazil thelytokous light form corresponds to *E. tabacivora*; and (iv) identify three new specific entities: the dark form, the New York dark form and the Texas light form, here described as *Encarsia gennaroi*, *Encarsia marthae*, and *Encarsia suzannae*, respectively.

In the current study, even though traditional multivariate analysis and MRA resulted in comparable outcomes, MRA represented a convenient framework for more immediate comparisons among specimens, effectively identifying diagnostic ratios that have not been used previously in *Encarsia* taxonomy and permitting an immediate inference on allometry. Between the two frequently used approaches, BPCA and MRA, we hence argue for the implementation of MRA methods for future revisionary studies. The diagnostic morphological traits and ratios identified in this study, in combination with the molecular markers provided for three of the five species, offer a tool not only for taxonomists but also for the scientific community at large, including ecologists involved in biological control of whiteflies. Furthermore, these three species are model systems

for the study of symbiotic interactions between parasitoid wasps and bacterial endosymbionts, prompting an array of biological, cytogenetic, genomic and transcriptomic investigations (e.g. Zchori-Fein *et al.,* 2001, 2004; Kenyon & Hunter, 2007; Perlman *et al.,* 2008, 2014; Chiel *et al.,* 2009; Harris *et al.,* 2010; Penz *et al.,* 2012; Mancini *et al.,* 2013, 2016). The present revision provides a stable taxonomic framework for such studies, hence increasing the appeal of this species complex for future study. Finally, considering the extensive area of distribution of the *E. pergandiella* species complex, spanning the entire American Continent, Australia and areas of introduction in the Old World, future studies aimed at characterizing more populations from different geographic areas by both molecular and morphometric tools, coupled with the study of biological traits, will be necessary to reveal the full extent of cryptic diversity in this complex of species.

Taxonomy of the *Encarsia pergandiella* complex

Diagnosis of the E. parvella *species group*

Tarsal formula 5-5-5; antennal formula 1, 1, 4, 2; F1 of female antenna without linear sensilla; slender forewing (usually more than 3× as long as wide) usually sparsely setose except for an asetose area around the stigmal vein; longest marginal fringe of forewing from half to as long as the width of the corresponding wing; mesoscutum with zero to six pairs of setae; each side lobe of mesoscutum with one to two setae; distance between anterior pair of scutellar setae subequal to that between posterior pair; propodeum usually smooth; third valvula from about one-half to as long as the second valvula and with rounded apex; male antenna with F5 and F6 partially fused.

Diagnosis of the E. pergandiella *species complex*

In addition to the above characters, the following features have been found on all specimens examined in the present study: F1 and F2 of female antenna without linear sensilla; midlobe of mesoscutum with reticulate sculpture and usually with five pairs of setae (sometimes with one to two additional pairs); each side lobe of mesoscutum with one seta; placoid sensilla on scutellum distantly placed, separated by 5–7× their maximum diameter; third valvula about 0.66× as long as the second valvula.

Key to the *E. pergandiella* species complex

Females

1. Ratio of second valvula to third valvula ≤1.51. Ratio of ovipositor to second valvula ≥1.69. Ovipositor approximately as long as midtibia (0.99–1.07, except for one specimen of Girault's series, having 0.94). Body colour yellow . . . . ***Encarsia pergandiella* Howard**
- Ratio of second valvula to third valvula ≥1.53. Ratio of ovipositor to second valvula ≤1.66. Ovipositor generally shorter than midtibia (0.70–0.98). Body colour variable 2

2 (1). Body colour mostly brown 3
- Body colour yellow 4
3 (2). One pair of setae on the sixth gastral tergite. Ratio of F3 length to midbasitarsus ≤0.62. Ratio of midtibia to forewing length ≥0.33. F1 0.75–0.98× as long as F3. Ratio of ovipositor to second valvula 1.37–1.62. Head and mesosoma mostly yellow, metasoma mostly brown . . . . ***Encarsia gennaroi* sp.n.**
-Two pairs of setae on the sixth gastral tergite. Ratio of F3 length to midbasitarsus ≥0.64. Ratio of midtibia to forewing length ≤0.33. F1 0.68–0.78× as long as F3. Ratio of ovipositor to second valvula 1.30–1.38. Body colour largely dark brown . . . . ***Encarsia marthae* sp.n.**
4 (2). Ratio of F5 length to third valvula ≤0.89. Ratio of midbasitarsus to forewing width ≤0.49. . . . . ***Encarsia suzannae* sp.n.**
- Ratio of F5 length to third valvula ≥0.92. Ratio of midbasitarsus to forewing width ≥0.49. . . . . ***Encarsia tabacivora* Viggiani**

Males

1. Two pairs of setae on sixth gastral tergite. Ratio of F1 width to midspur 0.86–1.21. Ratio of F4 length to midbasitarsus 0.88–1.03. Ratio of F3 length to midtibia 0.35–0.39. . . . . ***Encarsia marthae* sp.n**.
- One pair of setae on sixth gastral tergite. Ratio of F1 width to midspur 0.58–0.84. Ratio of F4 length to midbasitarsus 0.75–0.86. Ratio of F3 length to midtibia 0.28–0.35. 2
2(1). Ratio of midtibia to midbasitarsus 2.23–2.45. Dorsal part of mesosoma almost entirely brown . . . . ***Encarsia gennaroi* sp.n.**
- Ratio of midtibia to midbasitarsus 2.71–3.02. Dorsal part of mesosoma almost entirely yellow. . . . . ***Encarsia suzannae* sp.n.**

Encarsia gennaroi Pedata & Giorgini sp.n.

http://zoobank.org/urn:lsid:zoobank.org:act:4BA44DB0-E2C0-421E-AE26-D305C4F0804A
(Figs 7–9)
Encarsia pergandiella Howard: *sensu* Viggiani (1988): 160–162 (with male description); Polaszek *et al.* (1992): 386–387 (partly); Schmidt *et al.* (2001): 381–383; Schmidt & Polaszek (2007): 2212–2214.

Material examined. Holotype, USA: ♀, California, Berkeley, ex *Trialeurodes* sp. on *Helianthus* sp., 29.vii.2015 *(Giorgini)* (IPSP).
Paratypes, USA: 1 ♀, California, Berkeley, ex *Trialeurodes* sp. on *Helianthus* sp., 29.vii.2015 *(Giorgini)* (USNM); ITALY: 14 ♀, 8 ♂, laboratory culture (originated from individuals collected in Portici), ex *T. vaporariorum* on *P. vulgaris*, v.2004 (IPSP; USNM); 6 ♀, 8 ♂, laboratory culture (originated from individuals collected in Portici), ex *B. tabaci* on *V. unguiculata*, 10.ii.2014 (IPSP; USNM); SPAIN: 2 ♀, Canary Islands, ex *Trialeurodes* sp., 6.xii.2002 *(Polaszek)* (IPSP).
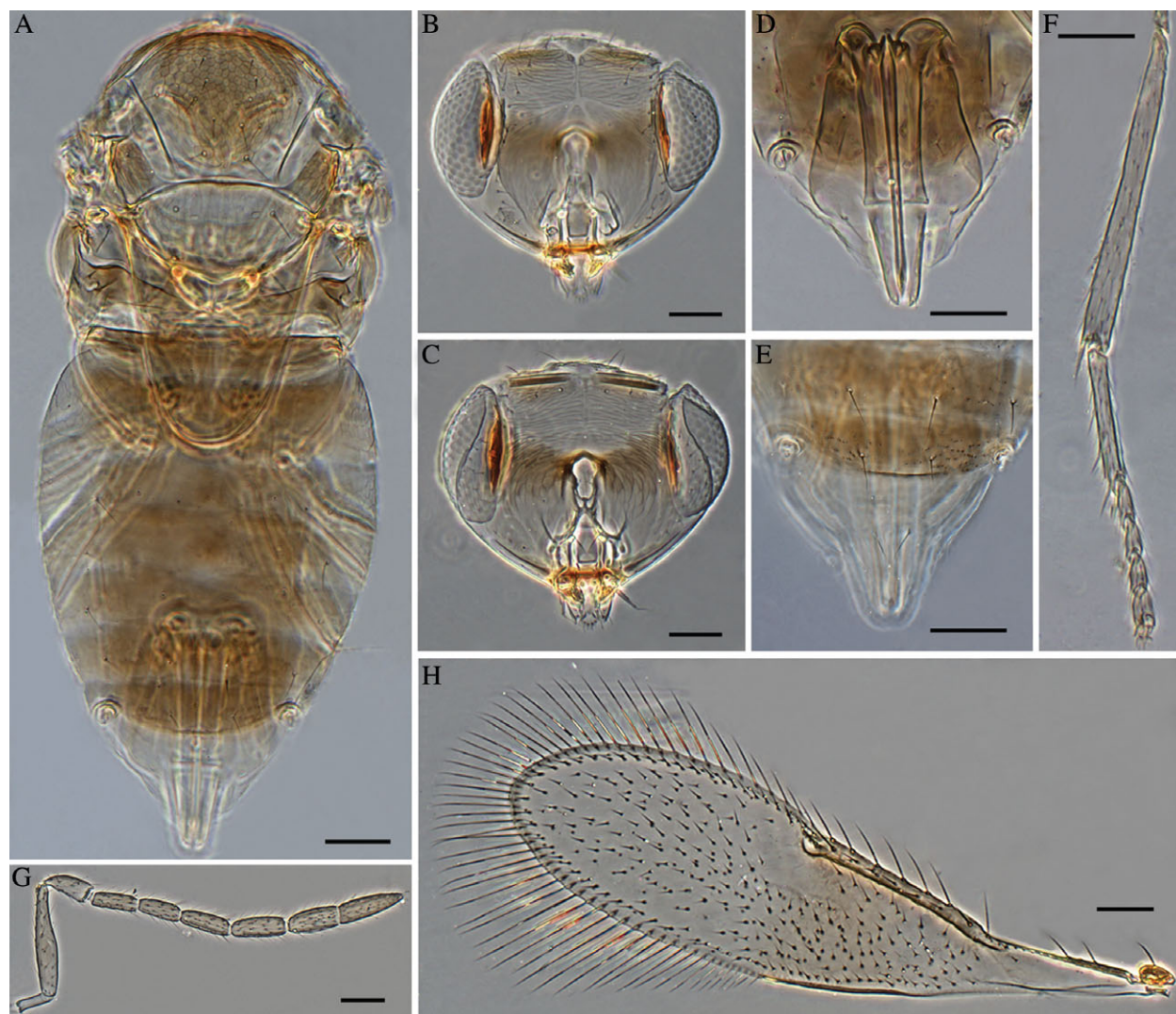
Fig. 7. *Encarsia gennaroi*. Female. (A) Mesosoma and gaster; (B) head, anterior view; (C) head, posterior view; (D) ovipositor; (E) setae on sixth tergite; (F) midtibia and tarsus; (G) antenna; (H) forewing. Scale bar = 50 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Other material. ITALY: 2 ♀, Palermo, ex *T. vaporariorum* on *Cyphomandra betacea* Send., slides 2a and 2b, ii.1988 *(Viggiani)* (MES); 1 ♀, Sperlonga, ex *T. vaporariorum* on *Conyza nandini* Bonnet [=*sumatrensis* (Retz) E. Walker], 06.iv.1982, *(Viggiani)* (MES).

Diagnosis. Females are characterized by a larger (on average) F1L/F3L compared with all other species (Table 2) and can be distinguished from: *E. marthae* by F3L/MB and MT/FWL, *E. pergandiella* by F3L/MB and OV/SV, *E. susannae* by MB/FWW, and *E. tabacivora* by the body colour and the combination of F1L/F2L and F5L/OV (Table 4, Figure S1). Males are characterized by slender F1 and by F1W/MB and F5L/MB compared with the other species of the complex and can be distinguished from *E. marthae* by F1W/FWW and F3L/MB, and from *E. susannae* by MB/MT (Tables 3, 4).

Description. Female (Figs 7, 9A, B). Head yellow except clypeus dark brown, and a narrow stripe across the occiput and post-ocellar bars behind lateral ocelli light brown (Fig. 7B, C). Antenna yellow. Mesosoma orange yellow except pronotum, a large inverted triangle on the midlobe of mesoscutum and axilla light brown. Legs yellow. Dorsal metasoma brown, except seventh gastral tergite yellow. Forewing hyaline and infuscated below marginal vein (Figs 7A, 9). Antenna (Fig. 7G) with pedicel slightly shorter than F1. F1 1.81–3.04× as long as wide, and 0.85–1.11× F2. F2 to F4 slightly increasing in length, each one 2.06–2.82, 2.05–3.33 and 2.32–3.06× as long as wide, respectively. F4 0.87–1.06× F5 and F5 shorter than F6 (0.72–0.90×). Flagellar segments with the following numbers of linear sensilla: F1, 0; F2, 0; F3, 1–2; F4, 1–2; F5, 2–3; F6, 2–3. Forewing (Fig. 7H) 3.33–3.90× as long as wide. Marginal fringe 0.63–0.83× wing width. Marginal vein with five to six

Fig. 8. (A–D) Male of *Encarsia gennaroi*; (E–H) male of *Encarsia suzannae*. *Encarsia gennaroi*: (A) mesosoma and gaster; (B) antenna; (C) head, anterior view; (D) head, posterior view. *Encarsia suzannae*: (E) mesosoma and gaster; (F) antenna; (G) head, anterior view; (H) head, posterior view. Scale bar = 50 µm. [Colour figure can be viewed at wileyonlinelibrary.com].

setae. Basal cell with one seta. Gastral tergites 1–7 with 0, 1, 1, 1, 2, 1, 2 pairs of setae respectively. Ovipositor shorter than midtibia (0.74–0.96×, except for the smallest individual among those examined, reared from *B. tabaci*, where OV/MT is 1.04), and 1.58–2.37× midbasitarsus, 1.37–1.62× second valvula and 2.30–2.75× third valvula length. Second valvula 1.55–1.90× third valvula. Midbasitarsus 2.07–3.09× midtibial spur (Fig. 7F).

Male (Figs 8A–D, 9C, D). Head orange brown with clypeus, stemmaticum and malar space brown, occiput dark brown (Fig. 8C–D). Antenna dark yellow. Mesosoma orange yellow with pronotum, an inverted triangle covering almost entirely the midlobe of mesoscutum, and axilla dark brown; scutellum bright yellow; ventral mesosoma predominantly brown. Legs yellow except for brown hind coxae. Metasoma largely dark brown except for yellowish sixth and seventh tergites, lighter brown

Fig. 9. *Encarsia gennaroi*: (A, B) female; (C, D) male. Scale bar = 100 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

on the ventral side (Figs 8A, 9C, D). Antenna (Fig. 8F) with pedicel shorter than F1 (0.70×). F1 subequal in length to F2 (0.90–1.08) and 2.13–2.97× as long as wide. F2–F4 subequal in length and each one 2.14–2.92, 2.47–3.15 and 2.40–3.15× as long as wide, respectively. F5–F6 partially fused. Flagellar segments with following numbers of linear sensilla: F1, 4–5; F2, 4–5; F3, 4–5; F4, 4–5; F5, 4–5; F6, 3–4. Forewing 3.28–3.76× as long as wide.

Distribution. USA: California, southern Texas, southern Florida (native distribution). Widespread in the Mediterranean Basin (Italy, France, Spain, Israel, Egypt) and the Canary Islands after repeated introductions as biological control agent; Australia.

Hosts. *Bemisia tabaci*, *Trialeurodes* spp., *Trialeurodes vaporariorum*.

Etymology. Named for Gennaro Viggiani, one of the most influential contributors to *Encarsia* taxonomy and biology, who introduced this species to Italy for biological control.

Remarks. This species is cosmopolitan because of its introduction as *E. pergandiella* from California to Europe (Italy and

Israel) in 1979 for biological control of *T. vaporariorum* (Viggiani & Mazzone, 1980; Rivnay & Gerling, 1987) and Egypt (Abd-Rabou, 2006). Its presence and distribution in Australia are probably a consequence of accidental introduction. *Encarsia pergandiella sensu* Gerling (1966), Viggiani (1988), Onillon *et al.* (1994), Liu & Stansly (1996a,1996b), Pedata & Hunter (1996), Heinz & Parrella (1994, 1998), Baldanza *et al.* (1999), Giorgini & Viggiani (2000), Pedata *et al.* (2002), Abd-Rabou (2006), Nannini *et al.* (2006), Mancini *et al.* (2013, 2016) and Zhang *et al.* (2014) all refer to this species.

Encarsia marthae Pedata & Giorgini sp.n.

http://zoobank.org/urn:lsid:zoobank.org:act:2C350074-91FE-498A-819E-3C9B5783BF34
(Fig. 10)

Material examined. Holotype, USA: ♀, New York, Ithaca, ex *T. packardi*, 29.ix.1992 *(Rose)* (UCR).

Paratypes, USA: 3 ♀, 5 ♂, New York, Ithaca, ex *T. packardi*, 29.ix.1992 *(Rose)* (UCR).

Additional material. USA: 2 ♂, lab culture, New York, Ithaca, Cornell Univ., vii.1991 *(Hunter)* (NHM).
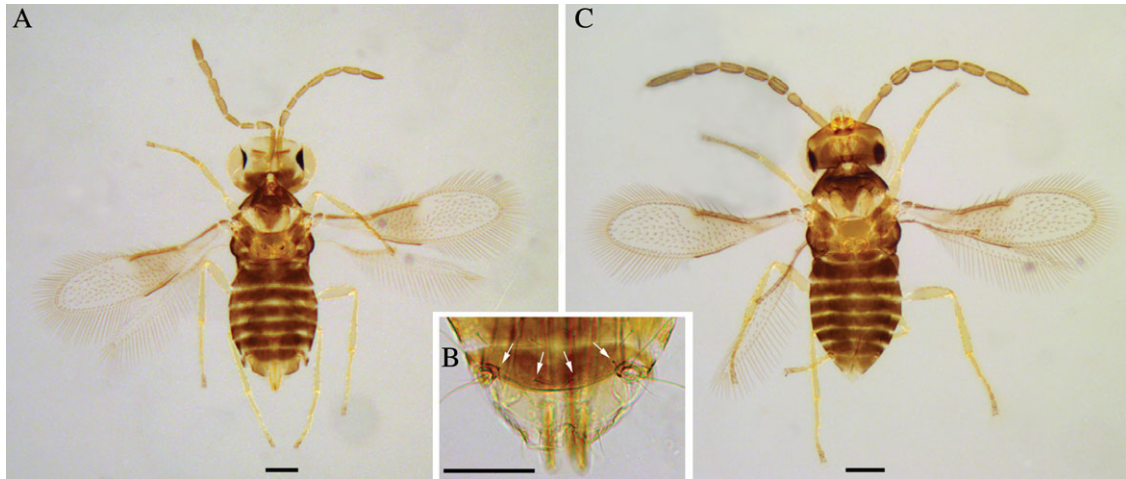
Fig. 10. *Encarsia marthae*: (A) female; (B) setae on sixth tergite (indicated by arrows); (C) male. Scale bar = 100 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Diagnosis. This very distinctively dark species is unique also in possessing two pairs of setae on the sixth gastral tergite in both sexes (Fig. 10B). Females can be separated from all other species by a combination of F3L/MT and SV/MT (Figure S1), and from *E. gennaroi* by F3L/MB and MT/FWL; *E. pergandiella* by MF/FWW, OV/SV, OV/MT, OV/MB and SV/TV; *E. susannae* by F4L/MS; and *E. tabacivora* by F6L/SV, MT/FWW, F1L/MT and OV/MT (Tables 2, 4). Males can be separated from the other species by F1W/MS, F4L/MB and F3L/MT, from *E. gennaroi* by F1W/FWW and F3L/MB, and from *E. susannae* by FWL/FWW and MT/MB (Tables 3, 4).

Description. Female (Fig. 10A). Head yellow except dark brown on malar space, a wide area on occiput, and post-ocellar bars behind lateral ocelli. Antenna brown. Mesosoma largely dark brown except for a posterior area on the midlobe and side lobes of the mesoscutum and scutellum which are light brown. Legs brown. Metasoma dark brown with a paler seventh gastral tergite. Forewing hyaline and strongly infuscated below the marginal vein. Antenna with pedicel slightly longer than F1 (1.1). F1 2.29–2.40× as long as wide, and distinctly shorter than F2 (0.87–0.90). F3 slightly longer than F2 and subequal to F4. F2 to F4 2.51–2.60, 2.46–2.90 and 2.51–2.92× as long as wide, respectively. F5 subequal in length to F4 (0.95–1.06×) and shorter than F6 (0.82–0.91×). Flagellar segments with the following numbers of linear sensilla: F1, 0; F2, 0; F3, 1; F4, 1–2; F5, 2–3; F6, 2–3. Forewing 3.52–3.57× as long as wide. Marginal fringe 0.65–0.68× wing width. Marginal vein with four to six setae, basal cell with one seta. Gastral tergites 1–7 with 0, 1, 1, 1, 2, 2, 2 pairs of setae respectively. Ovipositor shorter than midtibia (0.86–0.90×), and 1.89–1.99× midbasitarsus, 1.30–1.37× second valvula and 2.10–2.59× third valvula length. Second valvula 1.53–1.95× third valvula. Midbasitarsus 2.62–2.92× midtibial spur.

Male (Fig. 10C). Largely dark brown, except posterior of midlobe and sidelobes of mesoscutum and scutellum paler.

Antenna with pedicel shorter than F1 (0.70x). F1 distinctly shorter than F2 (0.84–0.91×) and 1.50–1.89× as long as wide. F2–F4 subequal in length and each one 1.89–2.47, 1.86–2.52 and 1.95–2.54× as long as wide, respectively. F5–F6 partially fused. Flagellar segments with the following numbers of linear sensilla: F1, 4–6; F2, 4–6; F3, 4–6; F4, 4–5; F5, 3–4; F6, 3–4. Forewing 3.30–3.62× as long as wide.

Distribution. USA: New York.

Host. Trialeurodes packardi.

Etymology. Named for Martha 'Molly' Hunter, who studied the sex allocation behaviour and a paternally transmitted selfish genetic element in her graduate work on this species.

Remarks. Even though *E. marthae* was considered conspecific with *E. pergandiella* by Myartseva & Evans (2008), the two species differ by several characters, in particular the relative length of midtibia and ovipositor, which in *E. marthae* is greater than one. The specimen labelled as 313 073 appears much lighter than the other ones and in the PCA on ratios is placed distantly from the other specimens yet close enough to be grouped within this species. *Encarsia pergandiella sensu* Kuenzel (1977), Hunter (1989a,1989b, 1993) and Hunter *et al.* (1993) refer to this species.

Encarsia pergandiella Howard
(Fig. 11A–C)
Encarsia pergandiella Howard 1907: 78. Syntypes ♀. USA: DC, Washington, 25.ix.1900 *(Pergande)*, ex 'Aleyrodes' [probably *Trialeurodes* sp.] on *Xanthium strumarium* L. (USNM, examined).
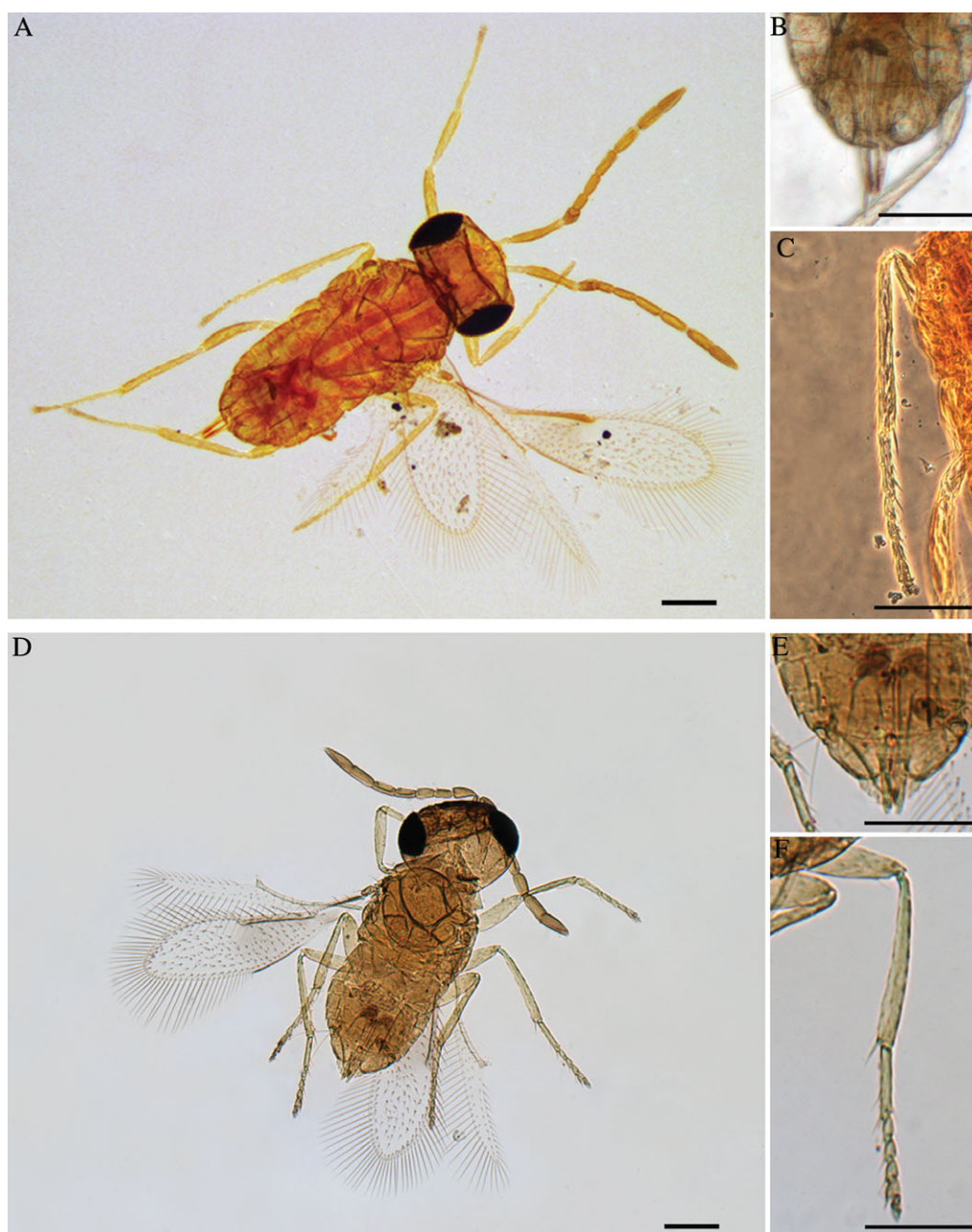*Encarsia versicolor* Girault, 1908: 53. Syntypes ♀ (INHS, examined). Synonymy by Gahan (in Peck, 1951: 438).

Fig. 11. (A–C) *Encarsia pergandiella* syntype; (D–F) *Encarsia tabacivora* holotype. *Encarsia pergandiella:* (A) whole specimen; (B) ovipositor; (C) midtibia and tarsus. *Encarsia tabacivora* holotype: (D) whole specimen; (E) ovipositor; (F) midtibia and tarsus. Scale bar = 100 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Aleurodiphilus pergandiellus (Howard): DeBach & Rosen (1981): 666, change of combination.

Encarsia pergandiella: Polaszek *et al.* (1992): 386–387 (partly); Myartseva & Evans (2008): 148–150 (partly).

Material examined. Syntypes, USA: 5 ♀, DC, Washington, ex 'Aleyrodes' [probably *Trialeurodes* sp.] on *X. strumarium*, 25.ix.1900 *(Pergande)*, (USNM).

Other material. Encarsia versicolor Girault, *syntypes,* USA: 9 ♀, Illinois, Urbana, ex *T. vaporariorum* on *Salvia*, pansy-geranium and other flowers in greenhouse, 4.iii.1908 *(Davis)* (INHS).

Diagnosis. Females of this species, as noted by Myartseva & Evans (2008), have the ovipositor as long as or slightly longer than the midtibia, differently from the other species of

the complex, which have the midtibia longer than the ovipositor, with very few exceptions. Two individuals of Girault's series seem to have an OV/MT <1.0, but in general they are in poor condition and many characters (including the shape of the antennae, which are collapsed) could not be reliably measured. However, ovipositor length relative to the midtibia of the other specimens of the same series suggests that they are conspecific with *E. pergandiella*. Further, *E. pergandiella* differs from the other species of the complex for SV/TV and OV/SV, from *E. gennaroi* by F3L/MB, from *E. marthae* by MF/FWW, OV/MT and OV/MB, from *E. susannae* by F4L/MS and MB/MS, and from *E. tabacivora* by F4L/F5L, F4L/F6L, F5L/OV and TV/MB (Tables 2, 4).

Description. Female (Fig. 11A–C). Head, meso- and metasoma and appendages yellow. Forewing hyaline and infuscated below marginal vein. Antenna with pedicel slightly longer than F1 (1.10×). F1 2.00–2.43× as long as wide and distinctly shorter than F2 (0.85–0.90×). F2–F4 slightly increasing in length, each one 2.36–2.64, 2.50–2.78 and 2.62–2.76× as long as wide, respectively (measured on Howard's types). F4 subequal to F5 (0.96–1.11×), and F5 shorter than F6 (0.82–0.90×). Flagellar segments with following numbers of linear sensilla: F1, 0; F2, 0; F3, 1; F4, 2–3; F5, 2–3; F6, 2–3. Forewing 3.19–4.08× as long as wide. Marginal fringe 0.71–0.84× wing width. Marginal vein with five to six setae. Basal cell with one seta. Gastral tergites 1–7 with 0, 1, 1, 1, 2, 1, 2 pairs of setae, respectively. Ovipositor shorter than midtibia (0.99–1.07×, except for one specimen of Girault's series, having 0.94), and 2.14–2.67× midbasitarsus, 1.69–1.90× second valvula and 2.24–2.81× third valvula length. Second valvula 1.31–1.51× third valvula. Midbasitarsus 2.24–2.99× midtibial spur.

Male. Unknown.

Distribution. USA: DC, Illinois.

Host. Aleyrodes (probably *Trialeurodes* sp.); *Trialeurodes vaporariorum*.

Remarks. Present on the slide containing the type material are five specimens of *E. pergandiella* plus one specimen of *Eretmocerus* sp. One individual is circled and identified as 'TH', probably standing for Holotype, but in the original description Howard did not designate any, and in the literature no lectotype was later selected. However, the individual indicated as the holotype is not properly cleared, the ovipositor measurements could not be taken and therefore this specimen was not included in the analysis. Myartseva & Evans (2008), in support of their concept of *E. pergandiella* (Howard's type material plus the New York material), included the presence of two pairs of setae on the sixth gastral tergite between the cerci. However, we found that Howard's syntypes bear only one pair of setae (with the exception of one individual having a supernumerary seta) and the presence of two pairs of setae appears to be restricted to specimens of the New York series. Girault (1908),

in describing *E. versicolor*, reported a colour pattern that does not correspond to the slide-mounted specimens, which appear uniformly yellow, similar to specimens of *E. pergandiella*. As the specimens do not appear to have been properly cleared (Figure S2), it seems unlikely that the original colour would not have been preserved. Furthermore, Girault included descriptions of males, but they could be located at either INHS or USNM or at the Milwaukee Public Museum, where cotypes (3 ♀ and 3 ♂, accession no. 11704, and 4 ♀ and 1 ♂, accession no. 1023/26105, respectively) should have been deposited. Therefore, until new data are obtained from the type locality, *E. versicolor* should be still considered a junior synonym of *E. pergandiella*.

Encarsia suzannae Pedata & Giorgini sp.n.

http://zoobank.org/urn:lsid:zoobank.org:act:F079A3CD-7688-4335-BBFD-FDDA74AE675A
(Figs 8, 12, 13)

Material examined. Holotype, USA: ♀, laboratory culture (originated from individuals collected in Texas, Weslaco), ex *B. tabaci* on *V. unguiculata*, 10.ii.2014 (IPSP).
Paratypes, USA: 4 ♀, laboratory culture (originated from individuals collected in Texas, Weslaco), ex *B. tabaci* on *V. unguiculata*, 10.ii.2014 (IPSP, USNM); 14 ♀, 8 ♂, laboratory culture (originated from individuals collected in Texas, Weslaco), ex *T. vaporariorum* on *P. vulgaris*, v.2004 (IPSP, USNM).

Diagnosis. Females can be distinguished from all the other species by a combination of MB/FWW and F5L/MS (Figure S1), and from *E. gennaroi* by MB/FWW; *E. marthae* by OV/MB, MT/MB and F4L/MS; *E. pergandiella* by F4L/MS and MB/MS, besides OV/MT (see *E. pergandiella* diagnosis); and *E. tabacivora* by F5L/TV and MB/FWW (Tables 2, 4). Males are distinguished from the other species by MT/MB and F4L/MT, from *E. gennaroi* by F1L/F1W and from *E. marthae* by FWL/FWW (Tables 3, 4).

Description. Female (Figs 12, 13A, B). Head, meso- and metasoma and appendages yellow. Forewing hyaline and very weakly infuscated below the marginal vein (Fig. 12G, 13). Antenna (Fig. 12F) with pedicel slightly longer than F1 (1.20×). F1 1.67–2.33× as long as wide, and distinctly shorter than F2 (0.74–0.91×). F2–F4 slightly increasing in length, each one 1.99–2.65, 2.18–2.93 and 2.05–2.86× as long as wide. F4 subequal to F5 (0.88–1.02×), and F5 shorter than F6 (0.74–0.85×). Flagellar segments with the following numbers of linear sensilla: F1, 0; F2, 0; F3, 1; F4, 2–3; F5, 2–3; F6, 2–3. Forewing (Fig. 12G) 3.32–3.65× as long as wide. Marginal fringe 0.68–0.84× wing width. Marginal vein with five to six setae. Basal cell with one seta. Gastral tergites 1–7 with 0, 1, 1, 1, 2, 1, 2 pairs of setae, respectively. Ovipositor shorter than midtibia (0.79–0.98×) and 2.07–2.41× midbasitarsus, 1.30–1.64× second valvula and 2.19–2.80× third valvula length. Second valvula 1.56–1.81× third valvula. Midbasitarsus 1.69–2.15× midtibial spur.

Fig. 12. *Encarsia suzannae,* female: (A) mesosoma and gaster; (B) ovipositor; (C) head, anterior view; (D) head, posterior view; (E) midtibia and tarsus; (F) antenna; (G) forewing. Scale bar = 50 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Male (Figs 8E–H, 13C, D). Head yellow with clypeus, stemmaticum and occiput brown (Fig. 8G–H). Antenna yellow. Mesosoma yellow except brown pronotum and an inverted triangle covering about half of the midlobe of mesoscutum; and axilla with a brown spot; scutellum bright yellow; ventral mesosoma yellow except for brown mesopleurae. Legs yellow. Metasoma largely brown except for yellow sixth and seventh tergites, pale on the ventral side (Figs 8E, 13C, D). Antenna (Fig. 8F) with pedicel shorter than F1 (0.80×). F1 subequal in length to F2 (0.90–1.02×) and 1.51–2.10× as long as wide; F2–F4 subequal in length and each one 1.83–2.31, 1.89–2.49 and 1.91–2.36× as long as wide, respectively. F5–F6 partially

fused. Flagellar segments with the following numbers of linear sensilla: F1, 4–6; F2, 4–6; F3, 4–6; F4, 4–5; F5, 3–4; F6, 3–4. Forewing 3.01–3.28× as long as wide.

Distribution. USA: Texas.

Host. B. tabaci (natural), *T. vaporariorum* (laboratory).

Etymology. Named for Suzanne E. Kelly for her dedication and contributions to *Encarsia* research over the last 20 years.

Fig. 13. *Encarsia suzannae*: (A, B) female; (C, D) male. Scale bar = 100 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Remarks. The Texas native *E. pergandiella* in Gould *et al.* (2008), *Encarsia pergandiella sensu* Schuster & Price (1996), Buckner *et al.* (2000), Bográn & Heinz (2002), Bográn *et al.* (2002) and Hunter *et al.* (2003), the sexual *E. pergandiella* in Zchori-Fein *et al.* (2004), Kenyon & Hunter (2007), Greenberg *et al.* (2008), Perlman *et al.* (2008), Chiel *et al.* (2009), Godfray (2010), Harris *et al.* (2010), Penz *et al.* (2012) and Perlman *et al.* (2014) refer to this species. Also, the first *Cardinium* endosymbiont genome sequenced (Penz *et al.,* 2012) is hosted by this *Encarsia* species, in which it induces cytoplasmic incompatibility (Perlman *et al.,* 2008, 2014).

Encarsia tabacivora **Viggiani**
(Figs 11D–F, 14, 15)

Encarsia tabacivora Viggiani (1985): 82. BRAZIL: *Holotype*, ♀, São Paulo, Campinas, ex *B. tabaci*, ix. 1979 *(Lourençao)* (UNLP, examined).
Encarsia bemisiae De Santis (1981): 37. Preoccupied by *Prospaltella bemisiae* Ishii, 1938.
Encarsia tabacivora Viggiani (1985): 82, replacement name for *Encarsia bemisiae* De Santis.

Encarsia tabacivora Viggiani, synonymized with *Encarsia pergandiella* Howard by Polaszek *et al.* (1992): 387.
Encarsia tabacivora Viggiani, revalidated by Myartseva & Evans (2008): 174.

Material examined. Holotype, BRAZIL: ♀, São Paulo, Campinas, ex *B. tabaci*, ix.1979 *(Lourençao)* (UNLP).
Paratypes, BRAZIL: 15 ♀, São Paulo, Campinas, ex *B. tabaci*, ix.1979 *(Lourençao)* (UNLP).

Other material. BRAZIL: 8 ♀, laboratory culture (originated from individuals collected in Minas Gerais, Sete Lagoas), ex *B. tabaci* on *V. unguiculata*, 10.ii.2014 (IPSP); 15 ♀, laboratory culture (originated from individuals collected in Minas Gerais, Sete Lagoas), ex *T. vaporariorum* on *P. vulgaris*, v.2004 (IPSP).

Diagnosis. This species can be distinguished from: *E. gennaroi* by body colour and the combination of F1L/F2L and F5L/OV (Figure S1); *E. marthae* by F6L/SV, MT/FWW and F1L/MT; *E. pergandiella* by F4L/F5L, F4L/F6L, F5L/OV and TV/MB; and *E. susannae* by F5L/TV and MB/FWW (Tables 2, 4).

Fig. 14. *Encarsia tabacivora*, female: (A) mesosoma and gaster; (B) ovipositor; (C) head, anterior view; (D) head, posterior view; (E) midtibia and tarsus; (F) antenna; (G) forewing. Scale bar = 50 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Female (Figs 11D–F, 14, 15). Head, meso- and metasoma and appendages yellow. Forewing hyaline very weakly infuscated below marginal vein. Antenna with pedicel slightly longer than F1 (1.10×). F1 1.77–2.32× as long as wide, and distinctly shorter than F2 (0.71–0.90×). F2–F4 slightly increasing in length, each one 2.15–2.85, 2.36–3.04 and 2.19–2.85× as long as wide, respectively (only measured on laboratory specimens). F4 slightly longer than F5 (0.85–0.95×) and F5 shorter than F6 (0.76–0.88×). Flagellar segments with the following numbers of linear sensilla: F1, 0–1; F2, 0; F3, 1; F4, 1–3; F5, 2–3;

Fig. 15. Female of *Encarsia tabacivora*. Scale bar = 100 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

F6, 2–. Forewing 3.40–3.94× as long as it is wide. Marginal fringe 0.67–0.91× wing width (0.67–0.89× for the laboratory specimens). Marginal vein with five to six setae. Basal cell with one seta. Gastral tergites 1–7 with 0, 1, 1, 1, 2, 1, 2 pairs of setae, respectively. Ovipositor shorter than midtibia (0.70–0.87×) and 1.70–2.18× midbasitarsus, 1.34–1.66× second valvula and 2.28–2.75× third valvula length. Second valvula 1.59–1.95× third valvula. Midbasitarsus 2.06–2.75× midtibial spur.

Male. Unknown.

Distribution. Brazil: Minas Gerais, São Paulo (native distribution); Mexico: Baja California; USA: Arizona, California, Texas (introduction range).

Host. *Bemisia tabaci*.

Remarks. Measurements of the width of flagellar segments were taken only on the specimens of the laboratory Brazil population because most of the antennae on De Santis' slides were collapsed or compressed. In the original description, De Santis (1981) indicated that the type material was composed of 15 ♂ paratypes in addition to a holotype. This is evidently a typographical error, as the type material consists exclusively of females and probably all populations of this species reproduce by thelytoky, as does the Brazilian population. This species was collected by Mike Rose at Sete Lagoas, Minas Gerais, Brazil, and was later introduced to the USA (southern Arizona, California, Texas) and to northern Mexico for the biological control of *B. tabaci* (Gould *et al.,* 2008). *Encarsia* sp. nr *pergandiella sensu* Goolsby *et al.* (1998) and Gould *et al.* (2008), *E. pergandiella*

sensu Zchori-Fein *et al.* (2001), Collier *et al.* (2002), Donnell & Hunter (2002), the asexual *E. pergandiella* in Zchori-Fein *et al.* (2004), and Kenyon & Hunter (2007) refer to this species.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12187

Figure S1. Diagnostic combination of best and second-best ratios found by LDA ratio extractor for: (A) *E. gennaroi* versus *E. tabacivora*; (B) *E. marthae* versus the other four species; (C) *E. suzannae* versus the other four species.

Figure S2. Female syntypes of *Encarsia versicolor*.

Table S1. Uncorrected *p*-distances based on COI data between *Encarsia* species. Interspecific distances are below diagonal, intraspecific distances are along diagonal, and standard errors are above diagonal.

Table S2. Measurements (μm) of morphological characters of *Encarsia pergandiella* species complex females. Mean (standard deviation) over range of variation. Abbreviations are explained in the text. *$n = 6$ for MF and OV. Different letters indicate significant differences (95%) by multiple range test (Tukey's HSD).

Table S3. Measurements (μm) of morphological characters of females of the three *Encarsia* species reared in the laboratory at constant temperature on *T. vaporariorum* or *B. tabaci*. Mean (standard deviation) over range of variation.

Abbreviations are explained in the text. Different letters indicate significant differences (95%) by multiple range test (Tukey's HSD).

Table S4. Measurements of morphological characters of *Encarsia pergandiella* species complex males. Mean (standard deviation) over range of variation. Abbreviations are explained in the text. Different letters indicate significant differences (95%) by multiple range test (Tukey's HSD). As for *E. gennaroi* and *E. suzannae*, males were reared in the laboratory at constant temperature.

Table S5. Mean ratios (standard deviation) over variation range of selected ratios of morphological characters of *Encarsia pergandiella* species complex females reared in the laboratory on *B. tabaci* or *T. vaporariorum* at constant temperature. Abbreviations are explained in the text. Different letters indicate significant differences (95%) by multiple range test (Tukey's HSD).

Table S6. Coefficients for the first four principal components (PC), eigenvalues and percentage of variance for backprojected data.

Table S7. Unstandardized linear discriminant coefficients for backprojected data.

Acknowledgements

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