etin of Entomological Research (2002) 92, 165–175

DOI: 10.1079/BER2001144

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Morphometric and molecular analysis of the *Encarsia inaron* species-group (Hymenoptera: Aphelinidae), parasitoids of whiteflies (Hemiptera: Aleyrodidae)

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Abstract

Several series of host-reared specimens of an *Encarsia* species, initially thought to be the cosmopolitan *Encarsia inaron* (Walker), were collected in the Azores Islands (Portugal). Subsequent morphometric analysis supported the presence of two species: *E. inaron* and a new species, described herein as *Encarsia estrellae* Manzari & Polaszek **sp. n.** *Encarsia estrellae* was reared from *Aleyrodes singularis* Danzig, *A.* ?*singularis*, and *Bemisia* sp. *afer*-group on several host plants. In addition, the D2 region of the 28S rDNA gene was sequenced in eight individuals belonging to these species, as well as single representatives of two closely related and one distantly related species. Phylogenetic analysis of these DNA sequences, together with 23 additional *Encarsia* sequences retrieved from the European Molecular Biology Laboratory (EMBL) and GenBank databases, further supported the specific status of *E. estrellae*, and the placement of *E. dichroa* (Mercet) in the *E. inaron* species-group. Additionally, *E. inaron* is redescribed and some taxonomic problems in the *E. inaron* species-group are discussed.

Introduction

Encarsia Förster (Hymenoptera: Aphelinidae) is a nomically difficult genus, which currently contains ut 280 described species (Polaszek *et al.*, 1999). *Encarsia* ies are mostly primary parasitoids of whiteflies miptera: Aleyrodidae) and armoured scale insects miptera: Diaspididae) – on which they are important ogical control agents – (Huang & Polaszek, 1998), but species are parasitoids of aphids (Hemiptera: Aphididae: Hormaphidinae) (Evans *et al.*, 1995) and a few species parasitize the eggs of Lepidoptera (Polaszek, 1991).

Although recent papers (Viggiani, 1985, 1987; Hayat, 1989, 1998; Polaszek *et al.*, 1992, 1999; Evans & Polaszek, 1998; Huang & Polaszek, 1998) have contributed to the identification of these parasitoids, reliable identification is still difficult for many species. This is for several reasons, e.g. their small size, diversity, and the existence of complexes of morphologically indistinguishable or hard to distinguish species (Polaszek *et al.*, 1999). The assignment of species-groups to *Encarsia* and the placement of the species within species-groups is also problematic, and a revision of the genus on a world basis is needed (Hayat, 1998). Approximately 29 species-groups are currently recognized

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within *Encarsia* but few of these groups can be recognized by discrete morphological characters. Indeed, for this reason, some species have been included in different groups by different authors (Viggiani & Mazzone, 1979; Heraty & Polaszek, 2000).

In September 1998 and June 2000 the second author and co-workers reared several series of an *Encarsia* species from several localities, and from different hosts and host plants, in the Azores Islands. After slide-mounting and examination with compound microscopy these were found to be morphologically extremely close to, if not the same as, the cosmopolitan *E. inaron* (Walker). Specimens were reared from *Aleyrodes singularis* Danzig, *A.* ?*singularis* (see note below under 'hosts'), and *Bemisia* sp. *afer*-group whiteflies (Hemiptera: Aleyrodidae). In this study we attempt to obtain objective views of species boundaries using principal component analysis (PCA) and canonical discriminant analysis (CDA) (Blackman & Paterson, 1986; Narusis, 1993; Chishti & Quicke, 1996; Añez *et al.*, 1997; Azidah *et al.*, 2000; Heraty & Polaszek, 2000). DNA sequence data were also used to test our findings and explore the support for existing species-groups. The D2 region of the rDNA gene was chosen for compatibility with the earlier study of Babcock *et al.* (2001). The *E. inaron* species-group currently contains 6 described species (http://cache.ucr.edu/~heraty/Encarsia.cat.pdf). In this catalogue Heraty & Woolley do not include *E. dichroa* (Mercet) in the *E. inaron* species-group, although Hayat (1998) does so. Our analysis of the *E. inaron* species-group also includes the first sequences for *E. dichroa* and *E.* sp. near *azimi* Hayat, from Cameroon. A morphological diagnosis of the group is provided by Hayat (1998), who, for Indian species, gives the following combination of characters: ovipositor shorter than mid tibia and basitarsus combined, mid tibial spur less than half-length of basitarsus, and clava 2-segmented. The applicability of this diagnosis was therefore tested. Finally, the new material is described as *E. estrellae* Manzari & Polaszek **sp. n.** and *E. inaron* is redescribed.

Materials and methods

Morphometric analysis

One hundred specimens of *E. inaron*, 59 females and 41 males, including the holotype and paratypes of *E. borealis* Huldeń (synonymized by Huang & Polaszek, 1998), and 11 specimens of *E. estrellae* (5 females and 6 males) were each measured using slide-mounted preparations and an ocular graticule. Forty-six variables (25 continuous and 21 discrete) were recorded for females, and 37 variables (22 continuous and 15 discrete) were recorded for males. Morphological terminology follows that of Hayat (1989) except the use of 'mesosoma' instead of 'thorax'. Material examined (table 1) belongs to the following collections: The Natural History Museum, London, UK (BMNH); Museo Nacional de Ciencas Naturales, Madrid, Spain (MNCN); Zoological Museum of the University of Helsinki, Finland (MZH). Continuous and discrete variables were analysed both separately and in combination. Measurements (continuous variables) used for PCA and CDA are illustrated in fig. 1 and listed in the legend.

Discrete variables were as follows: numbers of longitudinal sensilla (rhinaria) of the antennal flagellomeres (3 variables) (females only), numbers of setae on both left and right sides of mid lobe (2 variables) and side lobes (2 variables) of the mesoscutum, numbers of setae on both left and right sides of tergites 1–4 (8 variables) and all setae on tergites 5–7 (3 variables). Males and females were treated separately and the analyses were all carried out on untransformed data (Quartau, 1982; Blackman & Spence, 1994) based on correlation matrices using the statistical package SPSS (Narusis, 1993). Analyses of log-transformed data were also carried out and gave virtually the same results (not presented). Cases with missing values were treated in two different ways: replaced with the mean or excluded from the analysis. Since results of treating missing data in the two ways were almost the same, only the latter is presented.

DNA extraction, amplification and sequencing

Adults were killed directly in 70–95% ethanol. DNA was extracted from single specimens using the DNeasy Tissue Kit (Qiagen, Crawley, UK) with elution into 30 µl distilled water. Standard 50 µl polymerase chain reaction (PCR) was then carried out in a GeneAmp 9600 thermal cycler containing 1.0 µl DNA extract, 5 µl *Taq* buffer (1.5 mM $MgCl_2$), 1.5 U *Taq* polymerase (Roche), 10 nmol dNTPs (Amersham Pharmacia Biotech; APB, Amersham, UK) and 20 pmol of each primer. The D2 region of 28S rDNA was amplified using the following primers: forward 5′-GCG AAC AAG TAC CGT GAG GG-3′; reverse 5′-TAG TTC ACC ATC TTT CGG GTC-3′ (note, this product includes the smaller D3 region, which was not used in our analyses). GFX gel band purification (APB) was used to clean all PCR products, which were then sequenced in both directions with the same primers using *Big Dye* terminators at half recommended volumes on an ABI Prism 3700 automated sequencer. PCR conditions were 35 cycles of 95°C denaturation (30 s), 45°C annealing (30 s) and 72°C extension (1 min) with an initial denaturation for 2 min and a final extension for 4 min. The 28S-D2 locus was sequenced for four individuals of *E. estrellae* from a total of three localities in the Azores Islands, and four individuals of *E. inaron* from UK, Japan (two individuals) and India. Single individuals of two other probable members of the *E. inaron* species-group, *E. dichroa* and *E.* sp. near *azimi* (Cameroon) were also sequenced, plus a third unrelated species (*E. tricolor* Förster). Sequences have been deposited in the European Molecular Biology Laboratory (EMBL) and GenBank databases under accession numbers AJ305294–AJ305302 (table 2).

Phylogenetic analysis

Amplified D2 regions were between 454 and 457 bp in length. The sequences obtained, together with 26 additional ones retrieved from EMBL/GenBank (from Babcock *et al.*, 2001) including representatives of three related genera, were aligned by eye and also using the Clustal X programme with four sets of gap opening and gap extension penalties: 20:10, 15:6.66 (default), 10:5 and 5:1 (the default downweighting of transitions by 0.5 was used in all). Maximum parsimony analysis (MP) was then performed on the five multiple alignments using PAUP* (Swofford, 1998). Heuristic searches were carried out treating gaps as both missing data and informative with 1000 random additions followed by branch swapping using tree-bisection-reconnection (TBR) and unlimited maxtrees. In each alignment the bootstrap

e 1. Geographical and biological information of *Encarsia* specimens used in the morphometric analysis.

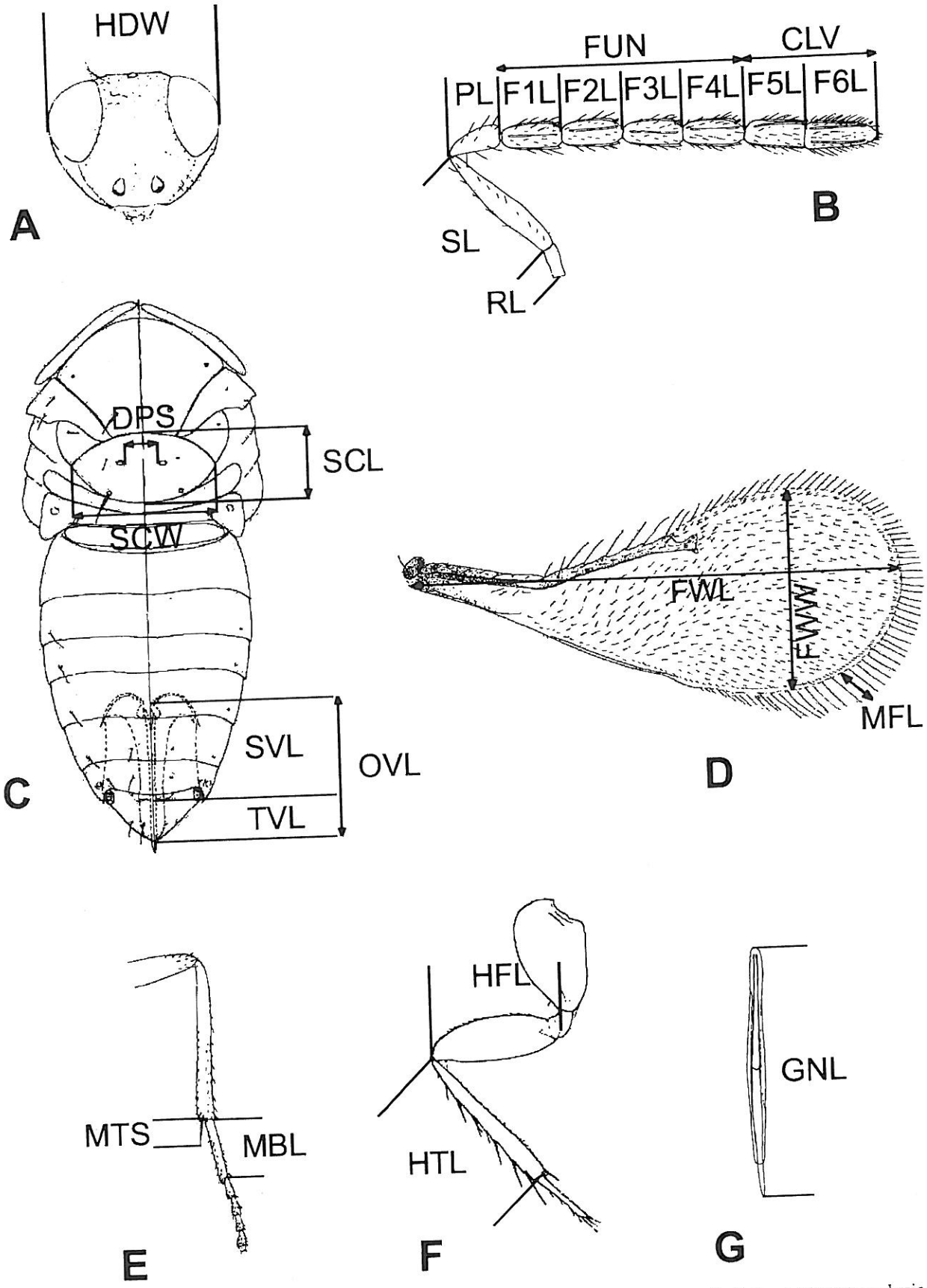
;ies	Locality	n	Host species	Host plant
ıaron	Afghanistan	2	Unknown	*Rosa* sp.
	Austria	1	Unknown	Unknown
	Bulgaria	1	*Siphoninus phillyreae* (Haliday)	*Crataegus* sp.
	Egypt	4	*Trialeurodes vaporariorum* (Westwood)	*Ricinus communis*
	Egypt	3	*Siphoninus phillyreae* (Haliday)	*Punica granatum*
	Egypt	2	Unknown	Unknown
	England	6	*Aleyrodes proletella* L.	*Brassica oleracea*
	England	3	*Aleyrodes proletella* L.	*Urtica dioica*
	England	2	*Aleyrodes proletella* L.	Unknown
	England	1	*Aleyrodes proletella* L.	Crucifer
	England	3	*Tetraleurodes ?hederae* Goux	*Hedera helix*
	England	1	*Pulvinaria vitis* (?misidentification)	*Vitis* sp.
	England	1	*Trialeurodes vaporariorum* (Westwood)	*Solanum* sp.
	England	1	Unknown	Unknown
	Finland	2	*Pealius quercus* (Signoret)	Unknown
	France	1	*Aleyrodes lonicerae* Walker	*Thalictrum minus*
	France	1	*Aleyrodes proletella* L.	*Chelidonium majus*
	India	1	Unknown	Unknown
	India	1	*Siphoninus phillyreae* (Haliday)	Unknown
	Iran	6	Unknown	Unknown
	Iran	3	*Siphoninus phillyreae* (Haliday)	Unknown
	Israel	2	Unknown	*Fraxinus* sp.
	Italy	7	*Siphoninus phillyreae* (Haliday)	Unknown
	Italy	3	*Pealius azaleae* (Baker & Moles)	*Robinia* sp.
	Italy	1	Unknown	Unknown
	Jordan	5	*Aleyrodes singularis* Danzig	*Citrus* sp.
	Jordan	1	Unknown	Unknown
	New Zealand	2	Unknown	Unknown
	New Zealand	2	*Siphoninus phillyreae* (Haliday)	*Gossypium hirsutum*
	Pakistan	7	*Bemisia tabaci* (Gennadius)	Unknown
	Pakistan	1	Unknown	*Lycopersicon esculentum*
	South Africa	2	Unknown	Unknown
	South Africa	1	Unknown	*P. granatum*
	Syria	1	Unknown	*Lactuca* sp.
	Syria	4	*Aleyrodes singularis* Danzig	Unknown
	Thailand	1	Unknown	*Pyrus communis*
	Turkey	4	*Siphoninus phillyreae* (Haliday)	*B. oleracea*
	Turkey	4	*Aleyrodes proletella* L.	*Sonchus* sp.
	Turkey	3	*Aleyrodes* sp.	Unknown
	Turkey	1	*Aleurothrixus floccosus* (Maskell)	*P. granatum*
	Venezuela	2	*Siphoninus phillyreae* (Haliday)	*Viburnum tinus subcordatum*
;trellae	Azores (Portugal)	1	*Bemisia* sp.	*H. helix canariensis*
	Azores (Portugal)	2	*Bemisia* sp.	*Ilex perado azorica*
	Azores (Portugal)	3	*Bemisia afer*-group	*Lysimachia nemorum*
	Azores (Portugal)	2	*Aleyrodes ?singularis* Danzig	*L. nemorum*
	Azores (Portugal)	3	*Aleyrodes singularis* Danzig	

·port for individual branches was found using 100 udoreplicates each of 100 random additions (Felsenstein, 5).

Results

Principal component analysis

ive components were extracted based on continuous iables for each analysis and biplots of all pair-wise ıbinations of these examined. Eigenvalues and weights first two principal components are presented in table 3. ıre 2a,b shows scatter diagrams of female and male *arsia* with respect to first and second principal ıponents obtained using continuous variables. In both sexes, the specimens can be seen to fall into two groups, with no overlap. Females and males of the two *Encarsia* species were projected on the first two principal components (PC-I and PC-II), which together accounted for 74.5% and 77.8% of the overall variance respectively. Length of marginal fringe and distance between placoid sensilla had the lowest contributions, and length of meta tibia, length of scutellum and length of fore wing had the highest influences on PC-I for male *Encarsia* (see table 3). Other variables had approximately equal contributions. For female *Encarsia*, length of third valvula, distance between placoid sensilla and length of marginal fringe had the lowest contributions, and length of funicle and length of flagellomeres 3 and 4 had the highest influences on PC-I (see table 3). As with males, other variables had approximately equal contributions.



g. 1. Measurements (continuous variables) used for principal component analysis and canonical discriminant analysis. A, head; B, ıtenna; C, mesosoma and gaster; D, fore wing; E, mid leg; F, hind leg; G, male genitalia. CLV, clava length; DPS, distance between acoid sensilla; F1L, flagellomere 1 length; F2L, flagellomere 2 length; F3L, flagellomere 3 length; F4L, flagellomere 4 length; F5L, ıgellomere 5 length; F6L, flagellomere 6 length; FUN, funicle length; FWL, fore wing length; FWW, fore wing width; GNL, male nitalia length; HDW, head width; HFL, hind femur length; HTL, hind tibia length; MBL, meso-basitarsus length; MFL, marginal nge length; MTS, mesotibial spur length; OVL, ovipositor length; PL, pedicel length; RL, radicle length; SCL, scutellum length; SCW, utellum width; SL, scape length; SVL, second valvifer length; TVL, third valvula length. A, F and G redrawn from Hayat (1998); B–E drawn from Polaszek *et al.* (1999).

e 2. Specimens of *Encarsia* used in the molecular phylogenetic analysis and their geographical and biological information.

	Locality	Host	EMBL/GenBank accession number
p. near *azimi* Hayat	Cameroon, Mbalmayo	Unknown	AJ305294
strellae sp.n. (az2)	Azores Islands, São Miguel	*Bemisia* sp. on *Viburnum* sp.	AJ305297
strellae sp.n. (az3a)	Azores Islands, Pico, Chão Vert	*Bemisia* sp. on *Rubia peregrina*	AJ305298
strellae sp.n. (az3b)	Azores Islands, Pico, Lagoa do Caiado	*Bemisia* sp. on *R. peregrina*	AJ305299
strellae sp.n. (az4)	Azores Islands, Pico, Lagoa do Caiado	*Bemisia* sp. on *Euphorbia stygiana*	AJ305300
lichroa (Mercet)	Spain, Granada	*Siphoninus* sp.	AJ305301
inaron (Walker)	England, London	*S. phillyreae* (Haliday)	AJ305295
inaron (Walker)	Japan, Sanyocho	*S. phillyreae* (Haliday) on *Punica granatum*	–
inaron (Walker)	Japan, Yamaguchi	*S. phillyreae* (Haliday) on *P. granatum*	–
inaron (Walker)	India, Tamil Nadu, Arrupukottai	Unknown	AJ305296
tricolor Förster	Azores Islands, São Miguel	Unknown	AJ305302

hese two sequences and the sequence of *E. inaron* from England are identical, so only the latter sequence was deposited in BL/GenBank databases.

Table 3. Eigenvalues and weights for first two principal components extracted based on continuous variables for females and males.

Variable	Principal components			
	I	II	I	II
	Females		Males	
Eigenvalues	16.76	1.88	15.84	1.28
HDW	0.0453	0.1961	0.0477	−0.2162
RL	0.0369	0.2701	0.0411	−0.0933
SL	0.0533	0.0971	0.0578	−0.0669
PL	0.0492	0.1338	0.0484	0.0922
F1L	0.0544	0.0155	0.0585	0.1067
F2L	0.0544	−0.0350	0.0581	0.1343
F3L	0.0569	−0.0211	0.0592	0.0681
F4L	0.0569	−0.0694	0.0590	−0.0002
FUN (females only)	0.0580	−0.0265	–	–
F5L	0.0536	−0.1420	0.0583	−0.1059
F6L	0.0509	−0.1781	0.0542	−0.2255
CLV	0.0541	−0.1656	0.0581	−0.1681
FWL	0.0551	−0.0011	0.0596	0.1209
FWW	0.0492	0.0713	0.0574	−0.0081
MFL	0.0346	−0.3034	0.0292	0.6468
SCL	0.0530	0.0871	0.0597	−0.1009
SCW	0.0483	0.1128	0.0545	−0.0811
DPS	0.0313	−0.0828	0.0305	−0.2064
HFL	0.0480	0.0648	0.0544	−0.0149
HTL	0.0539	0.1145	0.0617	−0.0247
MBL	0.0501	−0.0401	0.0543	−0.0546
MTS	0.0415	−0.2065	0.0510	0.1162
SVL (females only)	0.0418	−0.1375	–	–
TVL (females only)	0.0295	0.3189	–	–
OVL (females only)	0.0451	−0.0005	–	–
GNL (males only)	–	–	0.0504	0.2805

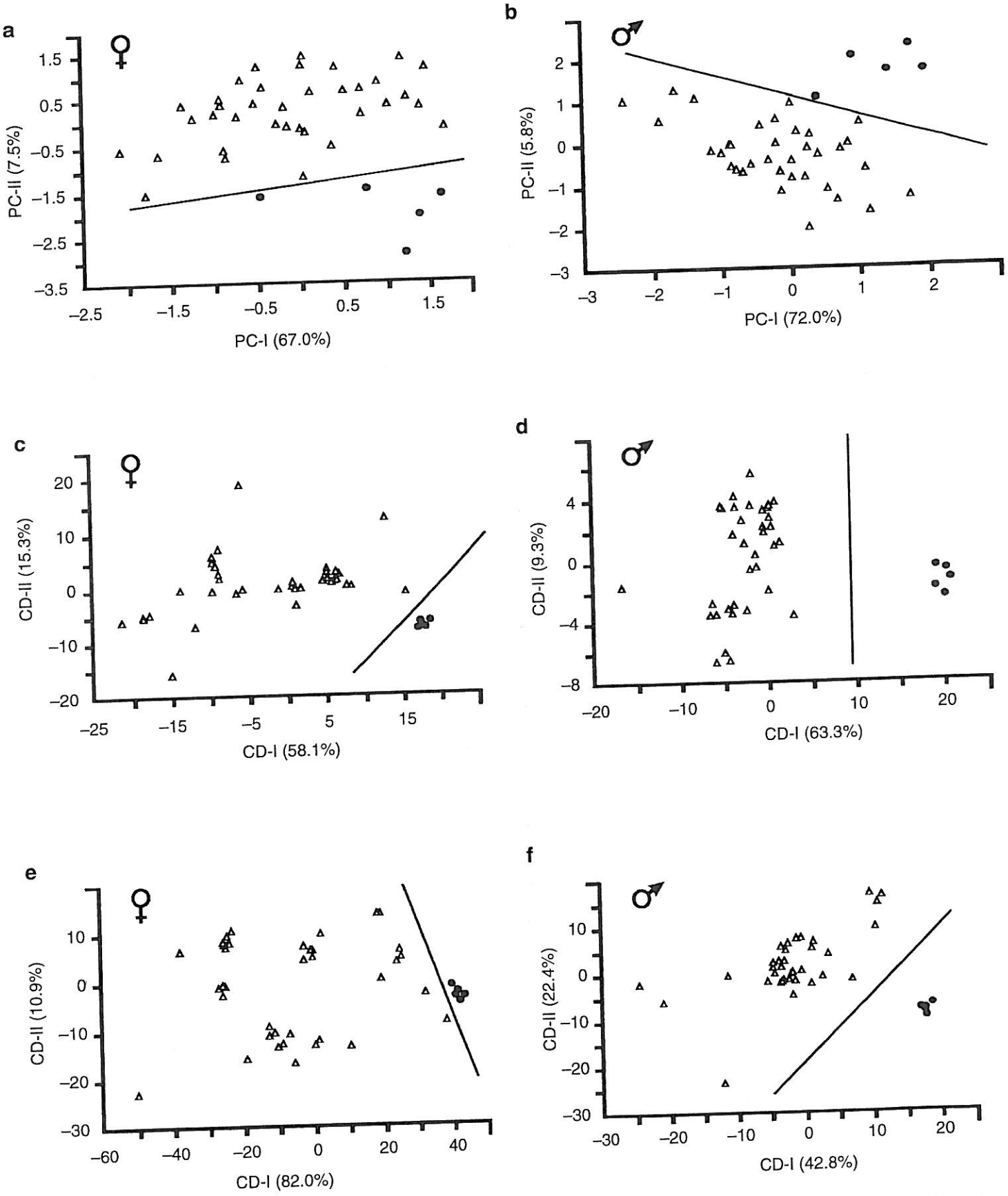
See fig. 1 for abbreviations.

Canonical discriminant analysis

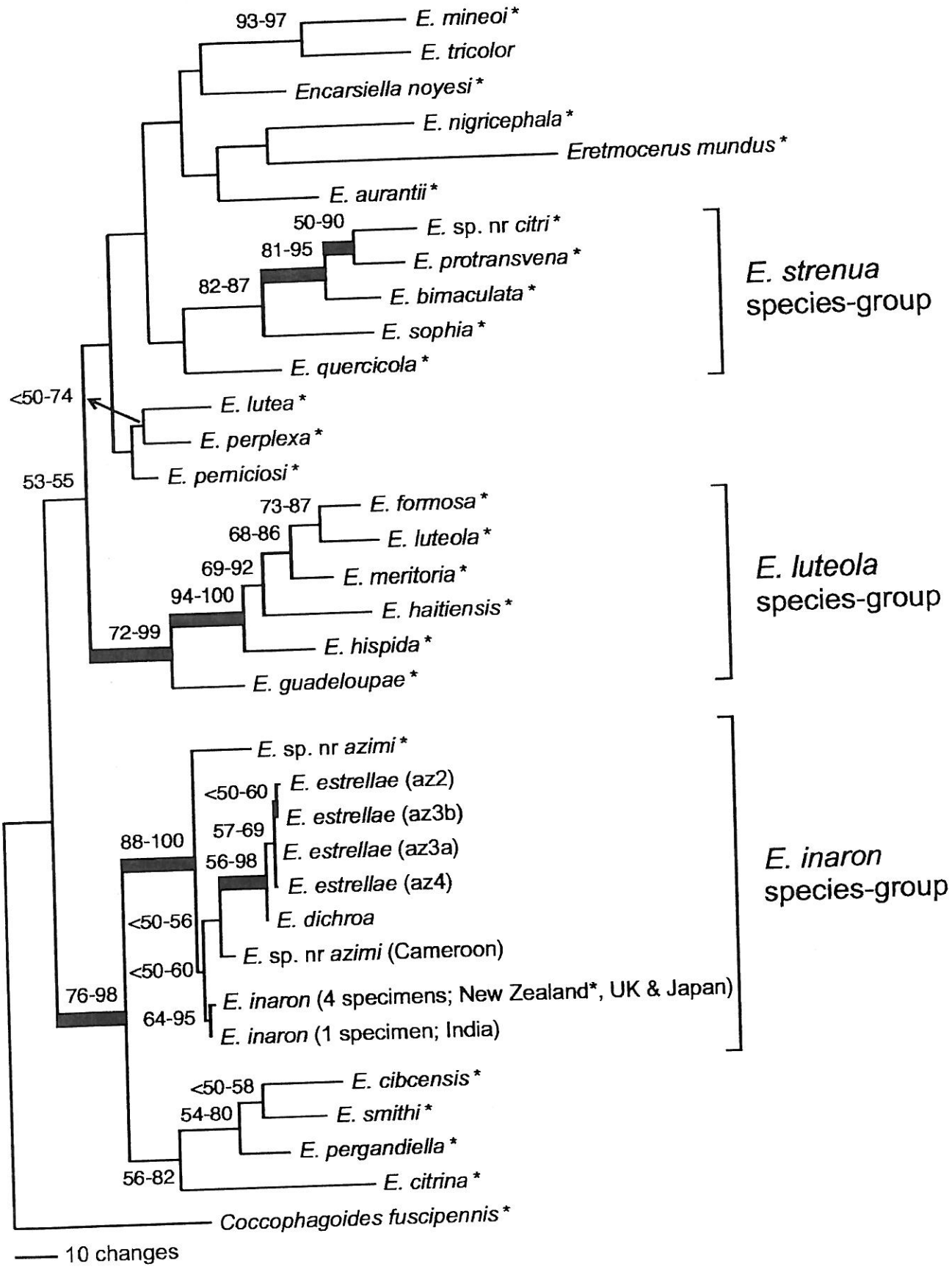
eparation between the putative species was more ced using canonical discriminant analysis of continuous ables, and combined continuous and discrete variables shti & Quicke, 1996). Based on continuous variables, ales and males were projected on the first and second nical discriminant function (CD-I and CD-II), which ther accounted for 73.4% and 72.6% of the original ance respectively (fig. 2c,d). Analysis based on combined nuous and discrete variables for both sexes gave no more separation than analysis based on continuous variables only (fig. 2e,f).

Phylogenetic relationships

Length of multiple alignments was 493–495, with 179–188 parsimony informative positions (gaps treated as informative). Maximum parsimony (MP) analyses of the 37 DNA sequences gave a total of 221 most-parsimonious trees (MPTs) from the five alignments and one of them is shown



g. 2. Results of morphometric analysis. a–b, plots of first two principal components based on continuous data for *Encarsia* specimens , females; b, males). c–d, plots of first two canonical discriminant function based on continuous data for *Encarsia* specimens (c, females; males). e–f, plots of first two canonical discriminant function based on combined continuous and discrete data for *Encarsia* specimens , females; f, males). Δ *E. inaron*, • *E. estrellae*.



3. One of 221 most parsimonious trees resulting from analyses of 28S-D2 rDNA sequence data (length = 866, R.I. = 0.641). Each ıence is from a single individual unless otherwise indicated. Branches are thickened when recovered in all MPTs from the five :iple alignments with gaps treated as both missing data and informative. Figures above branches show the range of bootstrap values ve 50%) for all analyses. An asterisk shows sequences from EMBL/GenBank databases (accession numbers: AF223366, 23369–70, AF254192–94, AF254207, AF254211, AF254213, AF254221, AF254225–27, AF254230–32, AF254234–37, AF254242, 54245–48, AF273667). See table 2 for accession numbers of new sequences (the inclusion of *Eretmocerus mundus* is here considerd to ı artefact).

n fig. 3. Those branches that are also recovered in all MPTs .re shown by thick lines. Within the *inaron* species-group, lespite the existence of clear morphological differences)etween *E. dichroa* and *E. estrellae*, these are found to be more :losely related than *E. estrellae* is to *E. inaron*. Our)hylogenetic analysis confirms that *E. dichroa* and E. sp. near *izimi* (Cameroon) belong to the *E. inaron* species-group. Moreover, our sequence of E. sp. near *azimi* (Cameroon) liffered from that of E. sp. nr *azimi* (Babcock *et al.*, 2001) by 1.0–5.4% (depending upon different alignments; gaps reated as missing data), and from this it could be inferred hat the specimens represent two different species.

Few sequence differences were found between ndividuals of *E. estrellae*: the average sequence distance)ased on different alignments was 0.54%, while the average equence distance between *E. inaron* and *E. estrellae* was 3.6% gaps treated as missing data).

The separation of *E. inaron* and *E. estrellae* was tested by onstraining them in maximum parsimony analyses to form clade (to the exclusion of *E. dichroa*). In this case, the hortest trees were between 6 and 20 steps longer than the nconstrained ones (gaps treated as informative), depending ipon the alignment. In four of the five alignments, these onstrained trees were significantly worse in Wilcoxon natched pairs sign tests implemented in PAUP* as the 'empleton test ($P = 0.003–0.0596$ for all possible tree-to-tree omparisons; in the fifth alignment $P = 0.1573–0.2888$).

Within the genus *Encarsia*, overall relationships were imilar to those found by Babcock *et al.* (2001), from whose tudy most of the sequences are derived. *Encarsia tricolor* ras recovered with *E. mineoi* Viggiani in all except one of the nost parsimonious trees, despite these two species having een placed in different species-groups (*E. tricolor* and *E. arvella* species-groups, respectively) (Heraty & Woolley, ttp://cache.ucr.edu/~heraty/Encarsia.cat.pdf). Although ne genus *Encarsia* is never recovered as monophyletic, onstraining it to be so resulted in most parsimonious trees nat were significantly longer in only one of the five ignments (gaps treated as informative).

Discussion

Both the morphometric and molecular analysis indicate nat the species from the Azores Islands is a new species, hich is described below together with a redescription of *E. aron* in the light of our analyses. The average sequence ivergence within *E. estrellae* was typical of the values)tained by Babcock *et al.* (2001) for other *Encarsia* species, nd the average sequence divergence between it and *E. aron* was within the range of between-species values.

The first principal component in general is thought to be rongly influenced by overall size variation and possible lometric effects (Woolley & Browning, 1987). The host nges for *E. inaron* and *E. estrellae* are variable (table 1), although some populations of *E. inaron* were restricted to a single host. Apart from the possible effect of hosts on general size, the discrimination of species was not affected by size (considering the separation based on other components). A canonical discriminant function appears to be the best means of separating these species. No single measurement ~~or ratio~~ provided a definite separation of the two species. *E. inaron* was most variable for both size and host range and the complexity of the separation is reflected by this variability.

The relative length of the marginal fringe, although playing an important role in *Encarsia* taxonomy, had almost no contribution in either sex to PC-I. Heraty & Polaszek (2000) also found that this character had little effect on PC-I for the *E. strenua* group.

Separation of E. estrellae *sp. n. and* E. inaron

Encarsia estrellae is morphologically very close to *E. inaron*, especially with respect to females. For females the main difference between the two species is the ratio of the third valvula length to the clava length. In *E. estrellae* the third valvula is less than 0.5 times as long as the clava, while in *E. inaron* it is at least 0.5 times as long as the clava. Male specimens can be distinguished by comparing the clava: the two claval segments are partially fused in *E. estrellae* (fig. 4c) but are separate in *E. inaron*. The main differences between the two species are summarized in table 4.

The *E. inaron* species-group, which was supported as a monophyletic group in this molecular analysis, has no defining suite of morphological characters. As already mentioned, this group has been characterized by having the ovipositor shorter than the mid tibia and basitarsus combined, a 2-segmented clava, and mid tibial spur less than 0.5 times basitarsus (Hayat, 1998). In this study, the latter character failed to place most *E. inaron* within the *E. inaron*-species group: in 59 female specimens, the mid tibial spur was on average 0.59 times as long as basitarsus. In addition, *E. dichroa*, which has a 3-segmented clava, was found to be a member of this group in the phylogenetic analysis.

All individual characters in DNA sequences, whether substitutions or insertion/deletion events, are prone to homoplasy, which means that single characters that define species-groups reliably are not available, however, two useful characters in the data can be emphasized. The following motif occurs 41 bases into the D2 region, and is found in all sequences of representatives of the *E. inaron* species-group (dashes represent gaps in the multiple alignment of all *Encarsia* species): AG(T/-)CCGCTTTG-GCTTCCGTGTGAA-CGCG. In positions 23–26 of this motif the AA-C was found only in representatives of the *E. inaron* species-group, and the insertion of a T in position 3 of this motif occurred only in *E. estrellae* and *E. dichroa* (no unambiguously aligned single character distinguished *E. estrellae* from all other *Encarsia* species).

Table 4. Summary of the main differences between *Encarsia estrellae* and *E. inaron*.

	E. estrellae	*E. inaron*
Female: third valvula length (TVL) / clava length (CLV)	TVL < 0.5 × CLV	TVL ≥ 0.5 × CLV
Male: fifth and sixth flagellar segments (F5 and F6)	Partially fused (fig. 4c)	Separate

Encarsia estrellae Manzari & Polaszek sp. n.

(fig. 4a–d)

cription. Female. Head dark yellow; occiput, areas behind ıtocellar bar brown; clypeus, malar space brown to dark brown. sosoma brown but pronotum, axillae and propodeum dark wn; middle of scutellum dark yellow to brown. Petiole brown, eral parts dark brown. Gaster brown to dark brown. Third vula yellow to dark yellow. Antenna dark yellow except scape, licel, anterior half of F5, F6 brown. Fore wings (fig. 4d) hyaline, htly infuscate below marginal vein. Legs yellow except tarsus brown. Antennal formula 1-1-4-2 (fig. 4b). Pedicel rter than F1–F6 individually. Flagellum with the following nbers of longitudinal sensilla: F1:2, F2:3, F3:3, F4:4, F5:4, F6:3. d lobe of mesoscutum, axillae and scutellum with distinctly culate sculpture, longitudinal on the central scutellum (fig. 4a). d lobe of mesoscutum with 4+2+2 setae. Each side lobe of soscutum with 3 setae. Placoid sensilla on scutellum relatively tantly placed, distance between anterior pair of scutellar setae ater than that between posterior pair. Fore wing 2.41 times as g as wide (77:32). Marginal fringe of fore wing short. Tarsal mula 5-5-5. T1–T7 with 0+0, 1+1, 1+1, 1+1, 4, 4 and 5 setae, pectively. Ovipositor shorter than mid tibia and basitarsus nbined (41:66), third valvula 0.36 times as long as second vifer (11:30).

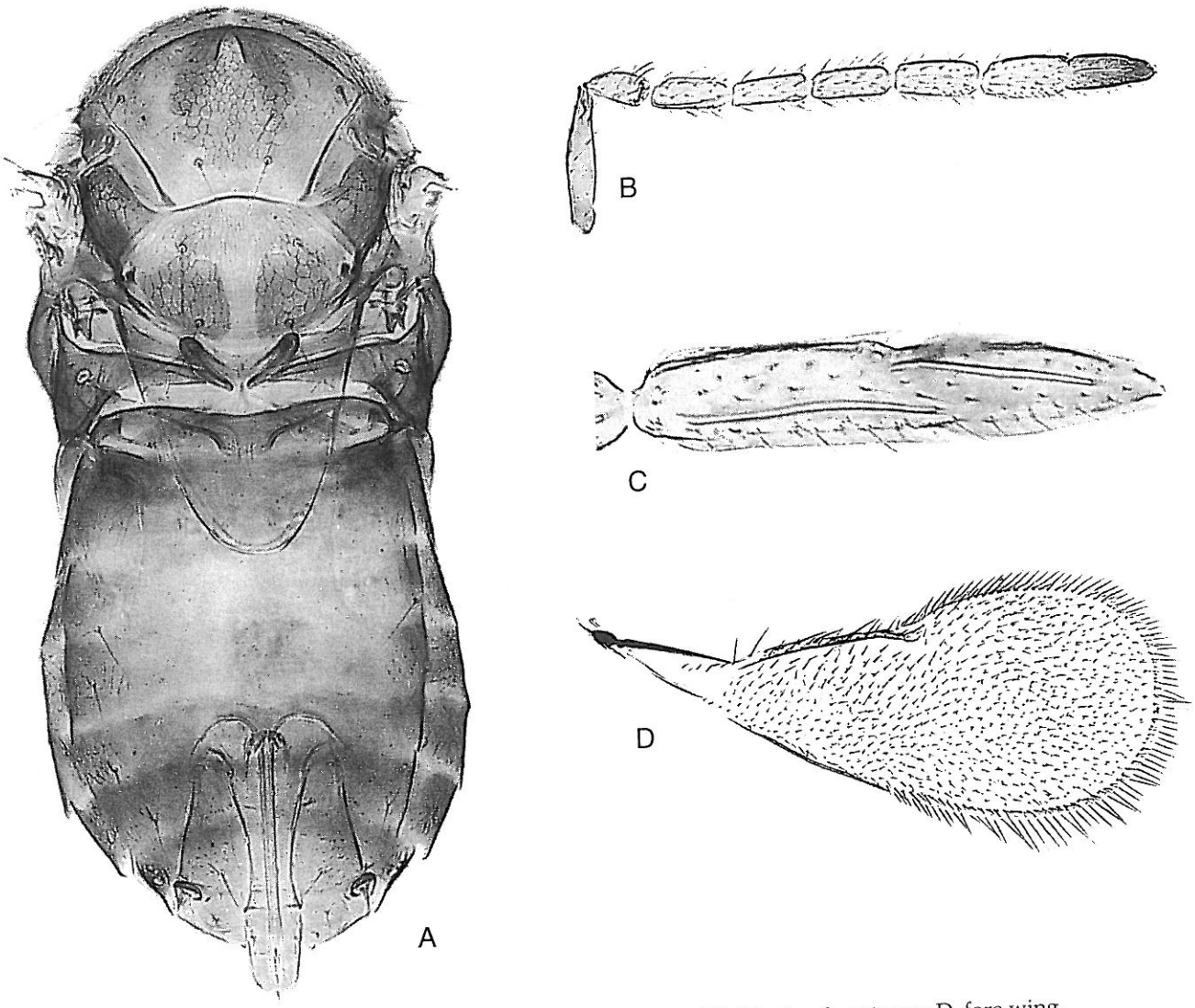
Male. Colour similar to female. Structural details essentially as for male, except ovipositor, and antenna with abundant longitudinal sensilla on all flagellomeres. The last two flagellar segments partially fused (fig. 4c). Male genitalia approximately as long as hind tibia.

Variation. In one female (ex *Bemisia afer*-group on *Hedera helix*), the gaster is largely pale, having only a single dark band on T6. Morphometrically this female and a (presumably conspecific) male agree with the remaining *E. estrellae* specimens, although no individual from this population was sequenced. Colour variation is well-documented in the closely-related *E. inaron* as a response to temperature experienced during development (Laudonia & Viggiani, 1993), and therefore could also be affected by altitude. These individuals are provisionally assigned to *E. estrellae*, pending further studies.

Species-group placement. *Encarsia inaron*-group.

Distribution. Azores Islands: Pico, São Miguel.

Hosts. Aleyrodidae: *Aleyrodes singularis* Danzig, *A.* ?*singularis*, *Bemisia* sp. *afer*-group. *Aleyrodes singularis* specimens from *Lysimachia nemorum* (Primulaceae) were positively identified by Dr J.H. Martin (BMNH). Specimens from the Azorean endemic *Euphorbia stygiana* (Euphorbiaceae) differ in some respects from *A. singularis*, and may represent a distinct, and possibly undescribed, species. In the north Atlantic islands the *Bemisia afer* group appears to be either highly diverse in terms of species, or highly morphologically variable within species. For this reason it has not been possible to identify to species-level some of the hosts of *E. estrellae*. The group is currently being studied in greater detail (J.H. Martin, BMNH, personal communication).



4. *Encarsia estrellae* sp.n. A, mesosoma and gaster; B, antenna, female; C, F5+F6 of male antenna; D, fore wing.

Material examined. Holotype ♀, AZORES, São Miguel, Lagoa Canarios, 715 m, 27.ix.1998, (E. Hernandez & A. Polaszek), ex *Aleyrodes singularis* on *Lysimachia nemorum* (BMNH). Paratypes 2♂, same data as holotype (BMNH). AZORES: São Miguel, Serra da Tronquiera, 2♀, 1♂, 26. ix.1998, (E. Hernandez & A. Polaszek) ex *Bemisia afer*-group on *Ilex perado azorica* (Aquifoliaceae) (BMNH). São Miguel, Sete Cidades, 1♀, 1♂, 27.ix.98, (E. Hernandez & A. Polaszek), ex *Bemisia* sp. on *Hedera helix canariensis* (Araliaceae) (BMNH). São Miguel, Serra da Tronquiera, 1♀, 1♂, 26.ix.1998, (E. Hernandez & A. Polaszek), ex *Aleyrodes singularis* on *Lysimachia nemorum* (BMNH). São Miguel, Serra da Tronquiera, 1♂, 26.ix.1998, (E. Hernandez & A. Polaszek), ex *Bemisia* sp. on *Viburnum tinus subcordatum* (Caprifoliaceae) (BMNH). Pico, Lagoa do Caiado 2♀, ♂, 27.vi.00 (A. Polaszek) ex *Aleyrodes ?singularis* on *Euphorbia stygiana* (BMNH) (not used in morphometric analysis).

Encarsia inaron (Walker)

Description. Female. Head, mesosoma and petiole brown to dark brown. Gaster variable, from largely pale to largely brown. Third valvula pale. Antenna brown. Fore wing hyaline except bases. Legs yellow except coxae. In dark specimens mid and hind femora brown. Antennal formula 1-1-4-2. Pedicel shorter than F1–F6 individually. Flagellum with the following numbers of longitudinal sensilla: F1:0–4, F2:1–4, F3:1–4, F4:2–5, F5:2–5, F6:2–4. Mid lobe of mesoscutum, axillae and scutellum with distinctly reticulate sculpture, longitudinal on the central scutellum. Mid lobe of mesoscutum with 8–12 setae. Each side lobe of mesoscutum with three setae. Placoid sensilla on scutellum distantly placed, distance between anterior pair of scutellar setae greater than that between posterior pair. Fore wing 2.09–2.50 times as long as wide (10:43–17.5:7). Marginal fringe of fore wing short. Tarsal formula 5-5-5: T1–T7 with 0–3+0–4, 0–3+1–4, 0–4+0–4, 0–5+0–4, 4–11, 3–8 and 3–4 setae, respectively. Ovipositor shorter than mid tibia and hind tarsus combined, third valvula 0.39–0.77 times as long as second valvifer (27:69–17:22).

Male. Entirely brown. Gaster sometimes pale. Structural details essentially as for female, except ovipositor, and antenna with abundant longitudinal sensilla on all segments. Antennomeres all separated. Male genitalia approximately as long as hind tibia.

Variation. See *E. estrellae* (above).

Species-group placement. *E. inaron*-group (= *E. partenopea*-group, *sensu* Viggiani & Mazzone, 1979).

Distribution. Europe, Africa, Asia, South America. Introduced into North America (Huang & Polaszek, 1998).

Hosts. Aleyrodidae: *Acaudaleyrodes rachipora* (Singh), *Aleurothrixus floccosus* (Maskell), *Aleyrodes lonicerae* Walker, *A. proletella* Linnaeus, *A. singularis*, *Asterobemisia carpini* (Koch), *A. paveli* (Zahradnik), *Bemisia tabaci* (Gennadius), *Bulgarialeurodes cotesii* (Maskell), *Pealius azaleae* (Baker & Moles), *P. quercus* (Signoret), *Siphoninus immaculatus* (Heeger), *S. phillyreae* (Haliday), *Trialeurodes vaporariorum* (Westwood).

Material examined. All specimens listed in table 1.

Acknowledgements

The authors are grateful to Roger Blackman (BMNH) for his comments on the morphometric analysis. Jon Martin (BMNH) kindly identified whitefly samples. Collecting in the Azores was funded by two Royal Society grants to A. Polaszek. Estrella and Alfredo Hernandez (Instituto Canario de Investigaciones Agrarias, Tenerife) assisted greatly with collection of whiteflies and parasitoids, and identification of host plants, in São Miguel. Paulo Borges (University of the Azores, Terceira) greatly facilitated access to several collecting sites in June 2000. Geoffrey Kibby (Digital Science) prepared fig. 4. S. Manzari is supported by the Ministry of Agriculture, Iran. R. Belshaw is funded by the Natural Environment Research Council.

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(Accepted 7 November 2001)

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