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The use of integrative taxonomy in determining species limits in the convergent pupa coloration pattern of *Aphytis* species

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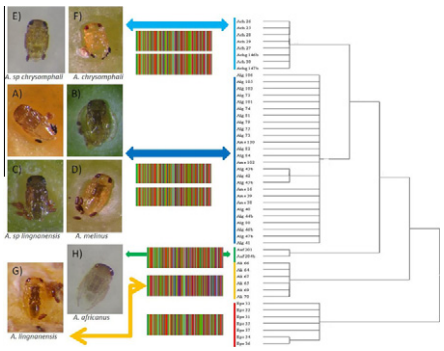
HIGHLIGHTS

- *Aphytis* species are used as natural enemies of California Red Scale in BC programs.
- Field surveys have revealed the presence of specimens with new pupa coloration pattern.
- Barcoding of COI and morphology -biological studies aid in the assignment to species.
- Convergent character plasticity (pupa coloration) has been unveiled in *Aphytis* species.
- A new integrative short-key is proposed for identification of *Aphytis* species in BC.

GRAPHICAL ABSTRACT



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ABSTRACT

Correct species identification in field surveys determines the success of natural enemies in biological control programmes. For such surveys, short-keys, mainly based on one or two morphological characters, are used as a quick-tool to ascertain species identification. However, when the whole character plasticity (the one used in the short-keys) has not been covered or is shared between species, some misidentifications could occur. *Aphytis* (Hymenoptera: Aphelinidae) are one of the most important natural enemies of armoured scales around the world. Pupa pigmentation pattern is the main character used in short-keys for species identification in field surveys in citrus production areas. However, field surveys in the Iberian Peninsula since 1998 have led to the misidentification of an increasing number of specimens that do not fit with the alpha-taxonomical keys for the described *Aphytis* species.

Integrative taxonomy has recently been used as a helpful tool in solving species limits and/or species assignments. In this study, we present the unification of molecular taxonomy (based on DNA barcodes) and traditional taxonomy, including biological studies, to clarify the species status of unidentified field-collected individuals of *Aphytis lingnanensis* group and *Aphytis chrysomphali* group, which parasitize California Red Scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae).

Our results (molecular, morphological and biological) show that these specimens belong to either *Aphytis melinus* DeBach or *A. chrysomphali* (Mercet) species, despite their differences in pupa pigmentation. We propose an integrative taxonomic key for assignment to the correct species for the use in field surveys on CRS in the Iberian Peninsula which takes into account the plasticity of pupa characters and a DNA barcode key for its use with damaged or immature specimens.

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1. Introduction

Molecular diagnostics are becoming a powerful tool in biological control programs, as they can provide accurate identification of natural enemies (Rugman-Jones et al., 2011), are able to track the establishment of released exotic natural enemies, deduce their population structure and monitor their impact on non-target organisms (reviewed in Gariepy et al., 2007). In this way, the use of a standard gene region, as proposed previously by Hebert et al. (2003) in the 'Barcoding of Life' consortium, will help taxonomy and taxonomists in the exploration of the biodiversity of world species, including those cryptic species or taxa that lack differential morphological characters, which render species separation difficult. Even more, the use of a standardized gene region benefits from the existence of universal primers which results in an economically affordable tool, which is altogether variable enough to successfully determine species boundaries (Hebert et al., 2003; Memon et al., 2006; Schmidt and Polaszek, 2007; Vaglia et al., 2008).

The genus *Aphytis* Howard (Hymenoptera: Aphelinidae) is a large, cosmopolitan group of minute wasps (comprised of about 130 species) that are usually yellowish or grayish and rarely exceeding one millimetre in length (Nikolaevna Myartseva et al., 2010; Rosen, 1994; Rosen and DeBach, 1979). All of the known species develop exclusively as primary ectoparasitoids of armoured scale insects (Hemiptera: Diaspididae) and are the most important natural enemies of these serious pests around the world (Bellows and Fisher, 1999). Despite its importance, its complex taxonomy has been responsible for misidentifications and has caused major setbacks in important biological control projects (DeBach, 1960; Rosen and DeBach, 1973, 1976). Identification and separation of *Aphytis* species is extremely difficult, mainly due to their minute size, the relative scarcity of reliable distinguishing characters, the common occurrence of sibling species, and the fact that a significant proportion of them are uniparental (Rosen and DeBach, 1979). DeBach (1964) described 16 characters commonly used in taxonomy, particularly with reference to females. Most of them refer to the number, shape, or ratio of some body structure. However, the last three characters refer to traits associated with pigmentation, such as the general colour of live adults; the degree of duskiness (melanisation) of the integument; its location on the body, head, antennae, legs and wings and the pigmentation of the pupa. Most of the characters related to number, size and shape change with body size and thus are not completely reliable. However, the pattern of pigmentation of the body and appendages is probably the least variable character in *Aphytis*, as it is surprisingly constant (DeBach, 1964; Rosen and DeBach, 1976, 1979). Similarly, the degree and pattern of pupal integument pigmentation, in some instances, provides the first clue to the distinctness of cryptic *Aphytis* species. Taylor (1935) was the first to notice differences in pupal pigmentation between closely related species of *Aphytis* (lemon-yellow pupae and dark pupae). Flanders (1953) and Compere (1955) separated *Aphytis lingnanensis* Compere from *Aphytis chrysomphali* (Mercet) on the basis of this character, and DeBach (1959) and Rosen and DeBach (1973) proposed the use of pupal pigmentation as a taxonomic character for species not readily separable by adult morphology. In fact, *Aphytis fisheri* DeBach, *Aphytis holoxanthus* DeBach and *Aphytis melinus* DeBach are considered sibling or near-sibling species, almost inseparable at the adult stage, but they are different in pupal pigmentation (Rosen and DeBach, 1979). Then, although pupal pigmentation is somewhat variable at times, it may be considered an important supplementary diagnostic character and may even serve as a convenient shortcut in field work to separate certain closely related species of *Aphytis* (DeBach, 1959; Muma and Selhime, 1966; Pina and Verdú, 2007; Quednau, 1964; Smith et al., 1997; Yasnosh, 1972).

California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), hereafter CRS, is the main armoured scale pest in citrus around the world (Bedford, 1998; Bodenheimer, 1951; Compere, 1961; Ebeling, 1959; Jacas et al., 2010; Talhouk, 1975). Biological control of this pest in the Iberian Peninsula is poorly established and is mainly carried out by *Aphytis* species, primarily *A. chrysomphali*, *A. melinus*, and recently, *A. lingnanensis* (Pekas et al., 2010; Pina, 2006; Sorribas et al., 2010). In this context, the *Aphytis* survey on CRS from citrus in the Valencia region (Spain) (Pina, 2006; Pina and Verdú, 2007; Vanaclocha et al., 2009), and most recently, from Andalusia (Spain) (Boyero et al., 2008) and Portugal (Pinto et al., 2010), has unveiled two new different *Aphytis* morphotypes based on their pupal colouration. One of them was included in the *lingnanensis* group of *Aphytis*, on the basis of the adult morphology, and has been named *Aphytis* sp. *lingnanensis* group (hereafter Asp1) (Pina, 2006; Pina and Verdú, 2007). This species exhibits a pupa with the head, thoracic sterna, appendages and wing pads darkened and, in some cases, the abdominal sterna as well (Fig. 1A–C); its exuvia reveals the same blackish structures. This morphotype has been increasing in number since its first appearance in 1999 in Alzira (Valencia, Spain). The other morphotype found in the Valencia region (T. Pina, unpublished data) exhibits the same black exuvia and pupal pigmentation but presents a fine black stripe between mid-coxae (Fig. 1E). This second morphotype, named *Aphytis* sp. *chrysomphali* group (hereafter Asp2), was included in the *chrysomphali* group based on adult morphology.

The combination of all morphological characters from the pupa, exuvia and adult for these two morphotypes does not correspond to any of the morphologies described by Rosen and DeBach (1979) or in the other recently published works related to *Aphytis* taxonomy (Abd-Rabou, 2004; Nikolaevna Myartseva et al., 2010; Rosen, 1994). This problem is of great importance as the short-keys used in field surveys are mainly based on one or two morphological characters. If one of these characters shows a great plasticity or has not been covered or even more, if it is shared between species, some misidentifications could occur, that is the problem encountered in this work. This mismatch in the taxonomic key of *Aphytis* species has directed our effort to the use of another character for the species assignment of Asp1 and Asp2 individuals.

The unification of alpha-taxonomy (traditional taxonomy) and molecular taxonomy (DNA barcoding) with complementary perspectives, such as phylogeography, comparative morphology, population genetics, ecology and behaviour, has rendered a new wave for species identification (e.g., Ciprandi Pires and Marinoni, 2010; Schlick-Steiner et al., 2010; Smith et al., 2008), which has been named Integrative Taxonomy (Dayrat, 2005). Due to the implications in the species assignment of Asp1 and Asp2 individuals in the biological control program of CRS in the Iberian Peninsula, we have performed an integrative taxonomy study to determine their taxonomic status. We analysed the DNA sequence data of the barcoding zone of the COI (cytochrome oxidase subunit I) gene, due to its success in the determination of species boundaries, and to keep a common reference for future studies in this economically important group. We have included a PCR-RFLP of the ITS region to ascertain a multiple approach to species identification (DeSalle et al., 2005) and also included a biological study to fit with the integrative taxonomy bases.

Therefore, the aim of this study is to clarify the taxonomic status of these specimens by molecular, morphological and biological approaches and to provide an integrative taxonomic key for the *Aphytis* species, which is important for the biological control of CRS in the Iberian Peninsula region.

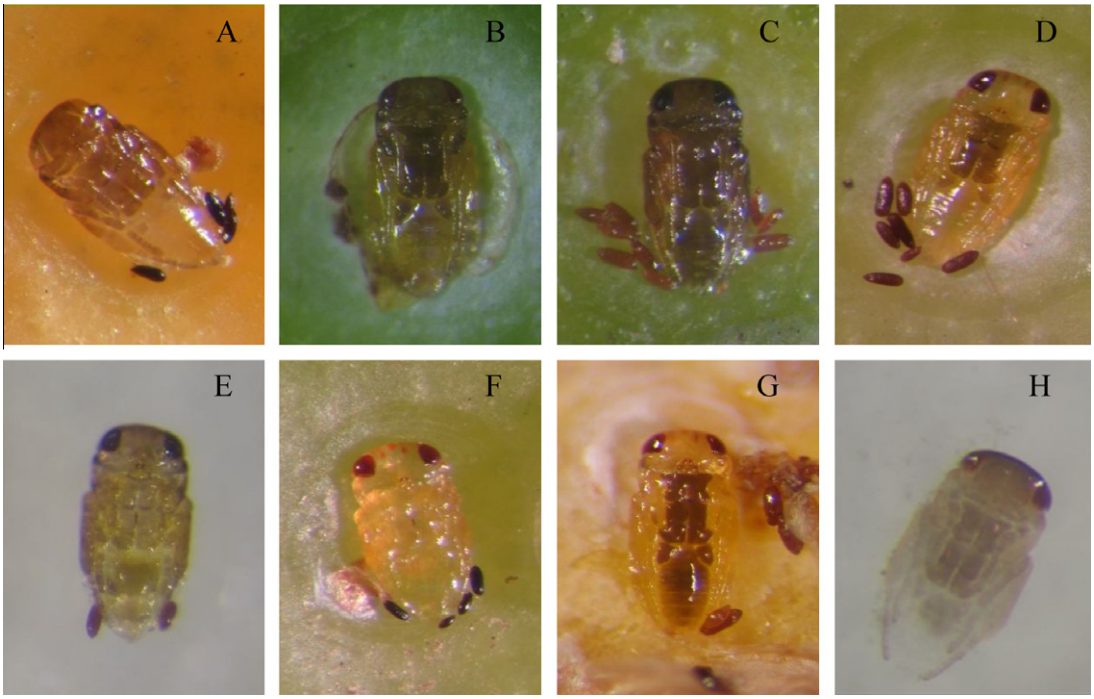

Fig. 1. Pupa pigmentation patterns found in the *Aphytis* species of this work. (A–C) *A. melinus* new pigmentation pattern; (D) *A. melinus sensu* DeBach (1959); (E) *A. chrysomphali* new pigmentation pattern; (F) *A. chrysomphali sensu* Rosen and DeBach (1979); (G) *A. lingnanensis*; (H) *A. africanus*.

2. Material and methods

2.1. Insect species

CRS and *Aphytis* species used in this work and their origin are listed in Table 1.

2.2. Morphological and biological approaches

CRS was used as a host for inbreeding crosses. CRS rearing was maintained on lemons (*Citrus limon* (L.) Burm f.) in bioclimatic chambers at 25 ± 1 °C, 70 ± 10% RH, 16L:8D h photoperiod (Pina, 2006). Lemons with *ad libitum* suitable CRS stages for *Aphytis* adults (Forster et al., 1995) were introduced into cardboard receptacles (8.5 cm high and 9 cm in diameter), with the upper side opened for ventilation and covered by gauze fixed with a rubber band. Lemons were fixed to the cardboard receptacle with white plasticine (Plastilina Jovi®; Jovi, SA, Barcelona). Honey drops were placed onto the inner walls of the receptacles and over the lemon surface as diet for *Aphytis* adults.

Performed crosses are outlined in Fig. 2. In all cases, individuals were field collected at the pupal stage, sexed and isolated according to pupa pigmentation (in red or green-eyed stage) and allowed to complete development in gelatine capsules to assure virginity of females. Half of the individuals of each morphotype were slide-mounted for morphological identification following Noyes (1982). Newly emerged Asp1 and Ame adults were randomly distributed into pairs in cups to perform the corresponding crosses, ♂Asp1 × ♀Ame ($n = 7$), ♀Asp1 × ♂Asp1 ($n = 3$) (Fig. 2). For ♀Asp1 × ♂Asp1 crosses, the F1 generation was used to obtain the F2 generation, which was used to obtain the F3 generation (by allowing self-sibling mating). Some of the F2 (Asp1 × Asp1) females ($n = 5$) were isolated to assess the form of sex determination. As no males were obtained from field samples of Asp2, females ($n = 3$) were isolated into cups to obtain the F1 generation. The Asp2-F1 offspring were used under the same conditions to obtain the F2 generation. In all cases, each couple or single female were allowed to parasitize one CRS-infested lemon for 72 h and were then transferred to another cup for the same parasitizing period until female death. Progeny of each cross were checked at the eleventh day age (red-eyed

Table 1
Species used in this work.

Orden	Group	Species	Initial host	Locality	Code
Hemiptera		*Aonidiella aurantii* (Maskell)		IVIA lab strain	Aau
Hymenoptera	*Lingnanensis*	*Aphytis melinus* DeBach	*Aspidiotus nerii*	Silla	Ame
			A. aurantii	Bétera	Ame
			A. aurantii	Moncada	Ame
		Aphytis sp. *lingnanensis* group	*A. aurantii*	Bétera	Asp1
			A. aurantii	Benimarxant	Asp1
			A. aurantii	Moncada	Asp1
		Aphytis lingnanensis Compere	*A. nerii*	Almazora lab strain	Ali
		Aphytis africanus Quednau	*Unknown*	Mpumalanga, South Africa	Aaf
	Chrysomphali	*Aphytis chrysomphali* (Mercet)	*A. aurantii*	IVIA lab strain	Ach
		Aphytis sp. *chrysomphali* group	*A. aurantii*	Moncada	Asp2
		Encarsia perniciosi (Tower)	*A. aurantii*	IVIA lab strain	Epe

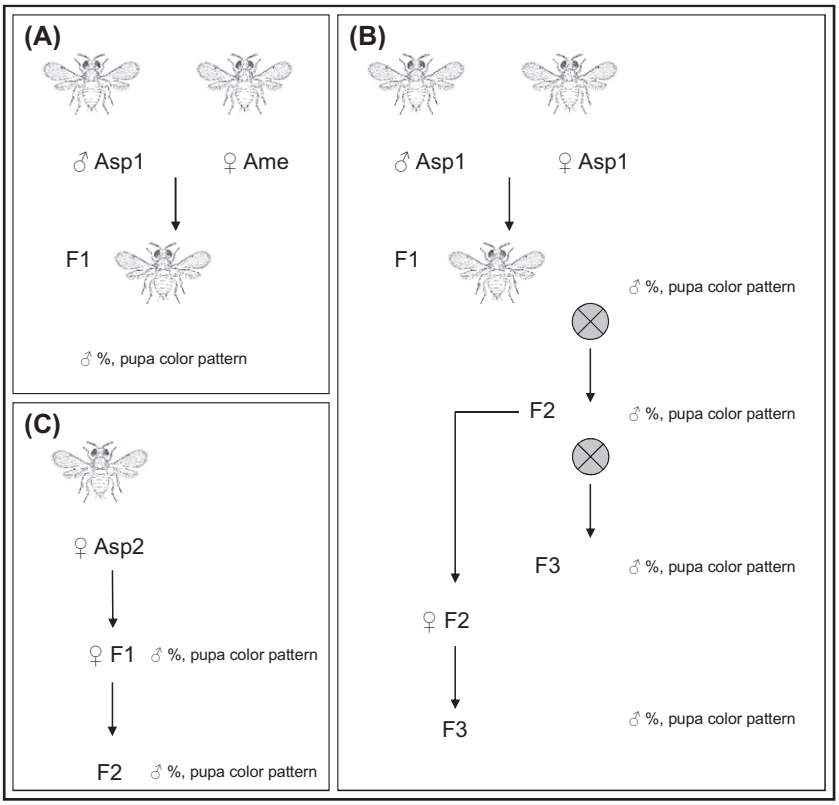

Fig. 2. Crosses performed with the different *Aphytis* spp.

and green-eyed pupal stage) by CRS dissection under a binocular microscope, and gender and pupal colouration of each individual was determined. All of these assays were performed in an environmental chamber at 25 ± 1 °C, 70 ± 10% RH, 16L:8D h photoperiod.

Voucher specimens have been deposited in the Entomology collection of the IVIA.

2.3. Molecular approach

Unless otherwise indicated, molecular protocols follow Sambrock et al. (1989).

2.3.1. DNA extraction

DNA was extracted from specimens listed in Table 1 by the "salting out" protocol (Sunnucks and Hales, 1996), adding fresh Proteinase-K at 100 μg/ml after tissue homogenization.

2.3.2. Amplification conditions

Two pairs of primers were tested, the universal ITS1 and ITS4 primers for the whole ITS region (White et al., 1990) and primers LCO1498 and HCO2198 for the barcoding region of COI (Hebert et al., 2003). Each primer pair was used in 20 μl volume reactions containing 300 nM dNTPs (Eppendorf AG, Hamburg, Deutschland), 1× DNA pol buffer (Biotools BandM labs S.A., Madrid, Spain), 3 mM $MgCl_2$ (Biotools), 0.75 μ DNA polymerase (Biotools), 10 pmol each primer (depending on marker), and 10 ng of total DNA. The amplification profile was one denaturation step at 94 °C for 2 min, 40 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 15 s, followed by a final extension at 72 °C for 150 s. Amplification was performed either in a Mastercycler® ep gradient-S thermal cycler (Eppendorf AG, Hamburg, Deutschland) or an AB 9700 GeneAmp PCR (Applied Biosystems, Foster City, CA, USA). PCR products were run on a 2% agarose D-1 low EEO (Pronadisa, Sumilab S.L., Madrid, Spain) gel in 0.5× TBE buffer, stained with ethidium bromide and visualized under UV light.

COI PCR products were purified with Sephadex G-50 prior to sequencing with BigDye v3.1 chemistry at the SCSIE Sequencing facility (University of Valencia, Spain). ITS PCR products were used for RFLP with *Afa*I, *Alu*I, *Dra*I and *Taq*I (Takara, Inc., Japan), as described by the manufacturer. Pooled restriction fragments were resolved on a 2% agarose gel in 0.5× TBE.

2.3.3. Sequence analysis

The STADEN package (Staden et al., 1998) was used to read and align chromatograms of each PCR product and to generate the consensus sequence of the COI fragment for each specimen. Alignment of consensus sequences of the individuals tested was performed with Genedoc (Nicholas et al., 1997), applying Blossum 62 matrix, with 8 and 4, gap aperture and extension penalty respectively. Sequence divergences were calculated using the Kimura 2-parameters model using Mega v5.0 (Tamura et al., 2011). Relationships among COI haplotypes were examined by reconstructing the phylogeny among the sequences by using the Neighbour-joining method (Saitou and Nei, 1987) as implemented in MEGA v5.0 (Tamura et al., 2011).

Nucleotide sequences have been deposited in GenBank under accession numbers (JQ083669 to JQ083717).

3. Results

3.1. Morphological and biological approaches

The adults of Asp1 and Asp2 field collected pupa were identified as *A. melinus* and *A. chrysomphali*, respectively, based on the morphological adult characters.

Five of the ♂Asp1 × ♀Ame crosses were fertile; the sex ratio obtained was almost 1:1, with an average of 56.4 % males. Similarly, the F1, F2 and F3 originated in the ♀Asp1 × ♂Asp1 crosses presented a male offspring average of 40.0 (n = 5), 43.3 (n = 150) and 41.2% (n = 354), respectively. In both cross types using Ame and Asp1 females, all the progeny obtained (F1, F2 and F3) exhibited the *A. melinus sensu* DeBach (1959) pupal pigmentation pattern as shown in Fig. 1D. When no males were offered to the Asp1 females, only males were obtained in the progeny (n = 333), confirming arrhenotokous parthenogenesis.

When Asp2 was considered, all F1 and F2 progeny exhibited the pupal pigmentation pattern described for *A. chrysomphali sensu* Rosen and DeBach (1979) as shown in Fig. 1F. Two of the Asp2 females gave F1 progeny that were exclusively males (n = 1 and n = 3), whereas the F1 progeny of one female produced only females (n = 10), with the F2 producing only females (n = 26); and the other female did not lay eggs. No crosses between the obtained males and females of the Asp2-F1 were conducted due to non-synchronic emergence.

3.2. Molecular approach

The amplified barcode zone of the COI gene in all of the species tested was about 700 bp. The COI amplicons were sequenced in both senses for the full length and in duplicate for almost all individuals. Alignments were unambiguous with no *indels* detected; one sequence from *Aphytis africanus* Quednau had a codon insertion, which was verified by re-sequencing. The absence of *indels* in the sequences corroborates that these sequences are derived from the mitochondrial DNA not from *numts* (nuclear mitochondrial pseudogenes; Bensasson et al., 2001; Williams and Knowlton, 2001). Overall, the sequences showed a strong A/T bias of about 75%. Sequence divergences, expressed as number of base substitutions per site, with an overall value of 0.094, ranged from 0.009 to 0.323 within groups (Table 2) and from 0.007 to 0.289 between groups (Table 3). Regarding the data of individuals with different pupa pigmentation patterns, the distances between morphotypes were of the same magnitude as those within each group (Table 2), which confirms that these individuals are closely related. The same distances were obtained in some of the generated offspring (data

Table 2
Overall mean distance over sequence pairs within groups.

Group	Distance
Ame	0.011
Asp1	0.011
Ach	0.011
Asp2	0.009
Aaf	0.323
Ali	0.007
Epe	0.018

Group codes are the same as for species in Table 1.

Table 3
Overall mean distance over sequence pairs between groups.

	Ame	Asp1	Ach	Asp2	Aaf	Ali
Ame						
Asp1	0.011					
Ach	0.088	0.087				
Asp2	0.085	0.085	0.007			
Aaf	0.282	0.284	0.291	0.293		
Ali	0.136	0.136	0.138	0.136	0.284	
Epe	0.142	0.140	0.137	0.135	0.289	0.018

Group codes are the same as species in Table 1.

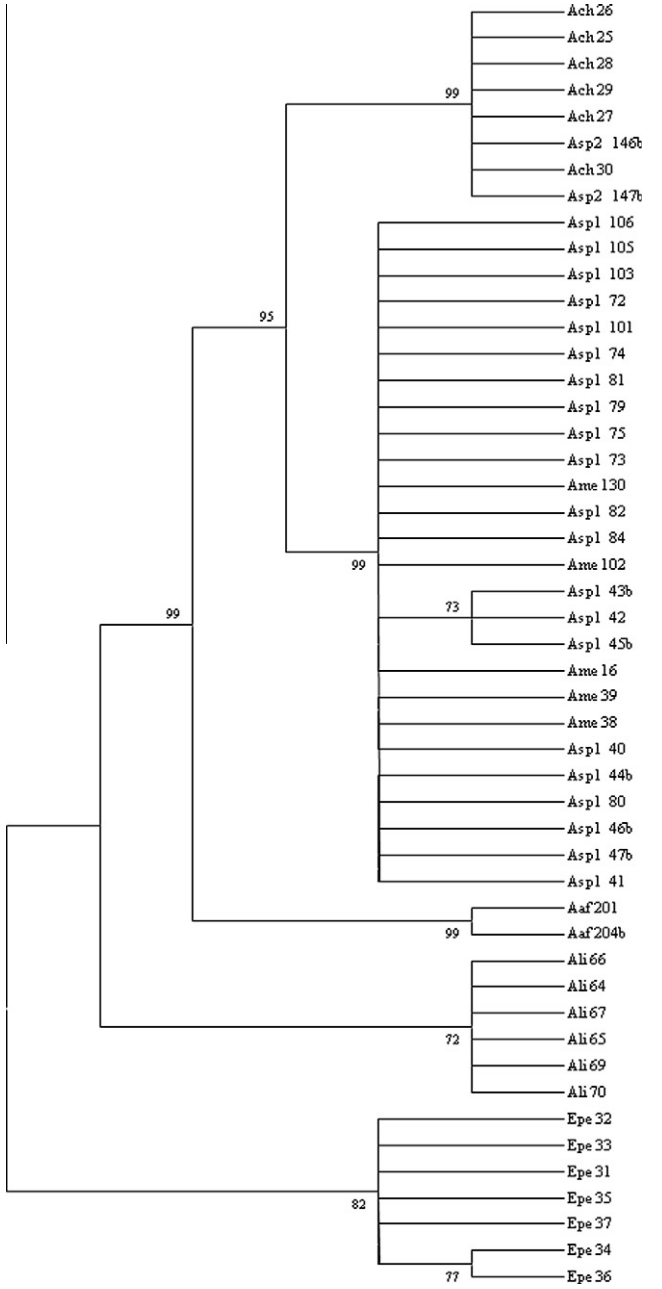

Fig. 3. Cladogram of relationships of *Aphytis* species including Asp1 and Asp2 individuals (see Table 1 for abbreviations).

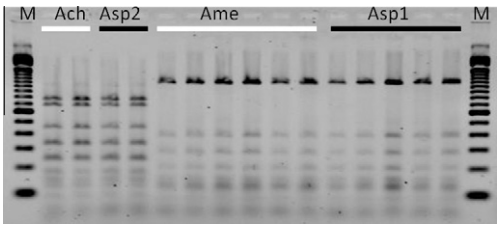

Fig. 4. Restriction fragment analysis of the amplified ITS gene region on *Aphytis* species. M, molecular weight marker 100 bp ladder (Invitrogen). Lines group RFLP patterns for selected *A. melinus* (Ame), *A. chrysomphali* (Ach), *A.* sp. *lingnanensis* gr. (Asp1) and *A.* sp. *chrysomphali* gr. (Asp2) individuals.

not shown), confirming the parental line origin of mitochondrial DNA. Of the total 706 positions in the final dataset, 251 were parsimoniously informative. The phylogenetic reconstruction confirms that all of the Asp1 and Asp2 individuals group together with individuals of *A. melinus* and *A. chrysomphali*, respectively (Fig. 3).

The ITS nuclear DNA region, analyzed by RFLP, confirmed the species status (of Asp1 and Ame specimens, and those of Asp2 and Ach), as no RFLP pattern polymorphism was observed between the Asp1 and Asp2 individuals with their counterparts *A. melinus* and *A. chrysomphali* (Fig. 4). These RFLP patterns could be used as a diagnostic marker for identifying all the used species, as each of the four *Aphytis* species tested (*A. melinus*, *A. chrysomphali*, *A. africanus* and *A. lingnanensis*) present a differential pattern (data not shown).

4. Discussion

Correct species identification in field surveys is of great importance in biological control programmes to ascertain the establishment of the introduced exotic species, to determine the balance between the native and the introduced, and even to detect new species of natural enemies or those that are invasive.

Pupa pigmentation is the main character used in the short-keys for *Aphytis* identification in field surveys of CRS biological control at citrus production areas (Pina and Verdu, 2007; Smith et al., 1997; among other references). The appearance of individuals showing Asp1 and Asp2 pupa patterns in surveys since 1999, which seemed to increase with the years (Boyero et al., 2008; Pina, 2006; Pinto et al., 2010; Vanaclocha et al., 2009), led us to perform this work to unveil the species nature of these specimens.

The adult morphology and species isolation tests conducted confirm that the *Aphytis* sp. *lingnanensis* group (Asp1) individuals belong to *A. melinus*, despite the fact that the pupa defined *sensu* DeBach (1959) exhibits "dark (blackish) pigmentation on the mid-thoracic sterna, less well-defined than in *A. lingnanensis*; abdomen clear (yellowish) ventrally; head, wing pads and appendages usually yellow; dorsal surface yellow" (Fig. 1D). Similarly, the *Aphytis* sp. *chrysomphali* group (Asp2) individuals belong to *A. chrysomphali*, despite the fact that the pupa pigmentation *sensu* Rosen and DeBach (1979) was defined as "entirely yellow, except for a longitudinal black line on the mesosternum" (Fig. 1F). A possible reason why this variation in pupa pigmentation is not recovered in the crosses performed could be related to the factor that this pigmentation could be a morphological trait influenced by developmental climatic conditions, as presented in other insect species (Bernardo et al., 2007; Gray and McKinnon, 2007). In this sense we observed that almost all the Asp1 and Asp2 individuals were collected during cold periods (end of autumn, winter and beginning of spring), which could be the responsible of this character plasticity observed (pupa colouration pattern).

We could not confirm *Wolbachia*-induced thelytokous parthenogenesis (Zchori-Fein et al., 1995), as we obtained males with two of the Asp2 field females. This result could be explained by an unequal transmission of the bacteria to the eggs; i.e., those eggs without *Wolbachia* developed into non-functional males, as demonstrated by Pina (2006) through the antibiotic treatment of *A. chrysomphali* females.

Sequence analysis confirmed that specimens identified at pupal stage as Asp1 are identical to *A. melinus*, and those identified as Asp2 are identical to *A. chrysomphali*, respectively, despite their intra-species differences in pupal pigmentation. The sequence analysis of the COI gene shows that sequence divergence between each *Aphytis* group (Ame *vs* Asp1, and Ach *vs* Asp2) is of the same magnitude that those of intra-group (see Tables 2 and 3), allowing us to assign a new pigmentation pattern for *A. melinus* and *A. chrysomphali* species. These distances and sequence characteristics are in concordance with values obtained with the other segment of the COI gene used by Monti et al., (2005) for species of the genus *Encarsia*, but are obtained in a standardized region that would allow a broad study of this economically important species group in a future.

In this sense, this study demonstrates that pupal pattern pigmentation, traditionally considered as a stable character to be used in taxonomy for a high number of species, and particularly for those of *Aphytis* genus, mainly *A. melinus* and *A. chrysomphali*, is not a completely reliable character under certain circumstances (i.e., climatic conditions). As has been presented here, two significantly different pattern of pupal pigmentation has been described for each species, *A. melinus* and *A. chrysomphali*; however one of the patterns is shared between both species requiring special attention to the black stripe in the mesosternum at the green-eyed stage pupa (Fig. 1C and E; Appendix A). The other three species that exhibit this dark pupal pigmentation pattern and that parasitize CRS are *Aphytis coheni*, *A. holoxanthus* and *A. africanus*. However, none of these three species are present in Spain; *A. holoxanthus* was introduced but never recovered (Jacas et al., 2006). In these species, differences in pupal pigmentation are found when the head, the thorax and the abdomen became infuscate (Quednau, 1964) at early pupal stages; however, at the late pupal stage (green-eyed), pigmentation pattern is quite similar, almost indistinguishable in all of them and very close to that reported in this work.

As reported here, the use of integrative taxonomy (considering morphological, biological, geographical and molecular characters) has allowed the correct identification of *Aphytis* species used in biological control programs of CRS. Moreover, the use of the barcoding region of the COI will allow an easy upgrading of this tree with other species and will help in the identification of damaged or immature specimens for which the morphological keys are not useful. The implementation of this study with another species will allow for improvement in the proposed short-key, which is needed for the correct identification of *Aphytis* species used in biological control programs of CRS in the studied area and in other citrus production regions (Appendix A). Further research should be conducted to obtain a non-genetically based answer to this character plasticity (i.e. climatic and rearing conditions).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocontrol.2011.11.004.

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