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Are generalist Aphidiinae (Hym. Braconidae) mostly cryptic species complexes?

STEPHANE A. P. DEROCLES[1,2], MANUEL PLANTEGENEST[1], JEAN-YVES RASPLUS[3], ALEXIA MARIE[1], DARREN M. EVANS[2], DAVID H. LUNT[2] and ANNE LERALEC[1]

[1]INRA, Agrocampus-Ouest, Université de Rennes 1, UMR1099 IGEPP, Rennes Cedex, France, [2]School of Biological, Biomedical and Environmental Sciences, University of Hull, Hull, U.K. and [3]INRA-UMR Centre de Biologie et de Gestion des Populations, CBGP, (INRA/IRD/CIRAD/Montpellier SupAgro), Montferrier-sur-Lez, France

Abstract. Aphidiinae are mostly composed of specialist parasitoids and the few species described as generalist are suspected to be composed of cryptic specialists, almost indistinguishable based on morphological characteristics. The use of molecular markers has proven to be a useful tool for revealing cryptic species complexes and here we use seven mitochondrial and nuclear gene fragments to study possible genetic differentiation among seven Aphidiinae generalists. Maximum likelihood (ML) trees and Bayesian Poisson tree processes (bPTP) models were conducted on each gene separately and on the seven genes together. The standard cytochrome *c* oxidase I barcode region appeared to be the most polymorphic and probably the best marker to reveal putative cryptic species. However, we showed with ML trees and bPTP models that a complementary use of mitochondrial and nuclear genes was the most relevant approach to reliably identify cryptic genetic clades in the Aphidiinae. Overall, most of the analysed generalist morphospecies were shown to be composed of subgroups related to the aphid host, some of them revealed as cryptic species by the species delimitation analysis. Further studies are needed to reveal the generality of this result in Aphidiinae.

Introduction

Parasitoid species account for a quarter of all insects (Godfray, 1994; Godfray & Shimada, 1999; Smith *et al.*, 2006, 2008) and represent an important component of worldwide biodiversity, as they are often a major cause of mortality for many insect species (Smith *et al.*, 2006). They are important ecosystem service providers and particularly help to regulate pest populations (Godfray, 1994; Godfray & Shimada, 1999). However, true parasitoid richness and host specificity are difficult to assess due to the large number of morphologically similar species that render their identification a difficult task (Smith *et al.*, 2008). Consequently, parasitoid specificity and richness may have been greatly underestimated (Bensch *et al.*, 2004; Westenberger *et al.*, 2004).

Correspondence: Anne Le Ralec, 65 rue de Saint-Brieuc, CS 84215, 35 042 Rennes Cedex, France. E-mail: anne.leralec@agrocampus-ouest.fr

Hymenoptera is by far the most species-rich insect order with respect to the number of parasitoids (La Salle & Gauld, 1992). Among them, the hyperdiverse Braconidae (with an estimated 40 000 species; Jones *et al.*, 2009) is exclusively composed of parasitoids (Gauld & Bolton, 1988), among which the subfamily Aphidiinae includes > 400 described species worldwide, all of them parasitizing aphids (Hemiptera: Aphididae; Sanchis *et al.*, 2001; Petrović *et al.*, 2014). Even though this group is extensively studied due to its important role in biological control of agricultural pests (e.g. Hawkins & Cornell, 1994; de Conti *et al.*, 2008), morphological identification of species in the Aphidiinae remains problematic as several species within genera are morphologically identical and consequently difficult to identify (Kavallieratos *et al.*, 2001; Tomanović *et al.*, 2003, 2007; Stanković *et al.*, 2015). Aphidiinae comprises numerous specialist species but some generalists are able to parasitize dozens of aphid species (Kavallieratos *et al.*, 2004; Starý, 2006; Tomanović *et al.*, 2009; Benelli *et al.*, 2014). However, as in other groups of parasitoids (e.g. Diptera: Tachinidae; Smith

et al., 2007), several species described as generalists are widely suspected to actually consist of cryptic species complexes (Tomić *et al.*, 2005; Desneux *et al.*, 2009; Žikić *et al.*, 2009; Barahoei *et al.*, 2011; Kos *et al.*, 2011).

A seminal study on cryptic biodiversity has taken advantage of the standardized use of a short section of a gene designed for species identification ('DNA barcoding'; Hebert & Gregory, 2005) to delimit species in a complex of cryptic species of Neotropical butterflies (Hebert *et al.*, 2004). This approach has been applied as well to parasitoid flies belonging to the Tachinidae family by Smith *et al.* (2006). The authors concluded that three of the previously considered generalist species are actually diversified complexes of highly host-specific cryptic species. Similarly, Derocles *et al.* (2012) suggested that the generalist species *Ephedrus plagiator*, *Lysiphlebus fabarum* and *Praon volucre* were probably cryptic species complexes but the sampling was too poor to confirm this. More recently, Petrović *et al.* (2015) also demonstrated that *L. fabarum* is composed of at least three phylogenetic lineages based on a maximum likelihood (ML) analysis of cytochrome *c* oxidase I (COI) sequences. However, the existence of so-called cryptic species is often due to a lack of diagnostic characters, emphasizing the need to find new features to delimit species among complex taxonomic groups (Al Khatib *et al.*, 2014; Baur *et al.*, 2014). In this context, molecular approaches are very useful to highlight putative cryptic species and taxa that may need further careful examination by taxonomists (see Bickford *et al.*, 2007 for a review).

In the present study, we used a molecular approach for seven generalist Aphidiinae species (called 'morphospecies' here), *Aphidius ervi*, *Aphidius matricariae*, *Aphidius urticae*, *Diaeretiella rapae*, *E. plagiator*, *L. fabarum* and *P. volucre*, to assess whether they may in fact be species complexes. A noninvasive DNA extraction protocol preserving morphological integrity was used to allow re-examination of morphological characteristics after sequencing. We used seven molecular markers (mitochondrial and nuclear including ribosomal), as we have previously shown that no single marker reliably identifies species of Aphidiinae (Derocles *et al.*, 2012). Indeed, several independent molecular markers are required to confirm the identification when dealing with recently divergent species or with species still capable of hybridization (Smith *et al.*, 2007). The objective of the study is to detect putative cryptic species using a combination of ML trees and a species delimitation analysis called the Bayesian Poisson tree processes model (bPTP; Zhang *et al.*, 2013). This approach delimits species based on the phylogenetic species concept (defining species in terms of monophyly without explicit regard to reproductive barriers; Bickford *et al.*, 2007). In addition to the seven generalist species listed, our dataset also included 22 additional species that are widespread in northwestern Europe to build more comprehensive ML trees in order to identify mismatches between morphological and molecular identification. With this molecular approach, we do not aim to delimit all new aphidiine species within the generalist morphospecies, but rather to demonstrate whether new undescribed species are common within generalist aphidiine parasitoids.

Material and methods

Parasitoid sampling

As part of routine sampling, plant samples infested with live and mummified aphids were collected in several locations in France (between 2007 and 2009), Chile (in 2009) and the UK (in 2012 and 2013). Live aphids were collected with a brush and stored in tubes with 96% ethanol. Mummified aphids were placed in a Petri dish and were transported to the laboratory for rearing. A strain of *P. volucre* comes from a breeding stock (**Viridaxis S.A., Gilly, Belgium, 2008). Aphid mummies were reared in the laboratory in a Petri dish at room temperature and we analysed 273 adult female parasitoids belonging to 29 morphospecies emerging from 37 aphid host species (up to eight host species per parasitoid species; Table 1).

After emergence, parasitoid specimens were stored in the laboratory in 96% ethanol at 20°C. Morphological identification of parasitoids was based on Starý (1973), Gardenfors (1986), Kavallieratos *et al.* (2001, 2005) and Tomanović *et al.* (2003, 2007). Morphological identification of aphids was based on Blackman & Eastop (1994, 2000, 2006).

DNA extraction and amplification

DNA was extracted from single specimens and amplifications were carried out on seven gene fragments following Derocles *et al.* (2012): COI, cytochrome B (CytB), rRNA 16S (16S), rRNA 28S (28S), long wavelength rhodopsin (LWRh), arginine kinase (ArgK) and elongation factor 1α (EF). Sequences of primers, hybridization temperature and sizes of amplified fragments are presented in Table S1. After extraction, specimens were again stored in 96% ethanol at 20°C. Vouchers were deposited at Agrocampus Ouest, 65 rue de Saint-Brieuc, CS 84215, 35042 Rennes Cedex, France.

Sequencing and sequence analysis

All PCR products were purified and both strands sequenced (Sanger technology; Sanger *et al.*, 1977). All samples and genes sequenced are presented in Table 1. We sequenced the seven genes for specimens from France. COI, CytB, LWRh and 16S genes were sequenced for specimens from the UK. Due to the low quantity of DNA available, only COI was sequenced for specimens from Chile (Table 1).

Sequences were edited using BIOEDIT 7.2.5 (Hall, 1999) and aligned with MAFFT 6.822 (default parameters, Katoh & Toh, 2010). Alignments were translated into amino acids using MEGA version 5 (Tamura *et al.*, 2011) to detect frameshifts or stop codons indicating pseudogenes. For LWRh, the 5′ intron was removed from the analyses because of large divergences in sequence impeding sequences alignment (Derocles *et al.*, 2012). Only the remaining 520 bp were used in the analysis. For ArgK, an intron after the 350 first 5′ bp was removed from the analyses for the same reason. Only the remaining 702 bp were used in the analysis.

Table 1. Samples included in this study.

Species[a]	Voucher numbers	Location	No. of individuals	Aphid host	No. of hosts	Plant species	COI	CytB	16S	28S	LWRh	ArgK	EF
A. aquilus Mackauer	1A, 1B, 1C	wF	3	*Betulaphis quadrituberculota* (Kaltenbach)	8	*Betula* sp.	+	+	+	+	+	+	+
A. avenae Haliday	1A, 1B, 1C	wF	3	*Sitobion avenae* (F.)	27	*Triticum aestivum*	+	+	+	+	+	+	+
A. colemani Viereck	1A, 1B, 1C	Belgium[b]	3	Unknown	40	Unknown	+	+	+	–	+	+	–
A. eadyi Starý, González & Hall	1A, 1B	wF	2	*Acyrthosiphon pisum* (Harris)	2	*Vicia faba*	+	+	+	+	+	+	+
A. eadyi	2A	wF	1	*Acyrthosiphon pisum*		*Medicago sativa*	+	+	+	+	+	+	+
A. ervi Haliday	1A, 1B, 1C	wF	3	*Acyrthosiphum pisum*	43	*Medicago sativa*	+	+	+	+	+	+	+
A. ervi	2A, 2B	eF	2	*Sitobion avenae*		*Triticum aestivum*	+	+	+	+	+	+	+
A. ervi	3A, 3B, 3C	wF	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	+	+	+	+
A. ervi	4A, 4B	Chile, T	2	*Acyrthosiphum pisum*		*Pisum sativum*	+	0	0	0	0	0	0
A. ervi	5A, 5B, 5C	UK	3	*Acyrthosiphum pisum*		*Pisum sativum*	+	+	+	0	+	0	0
A. ervi	6A, 6B, 6C	UK	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	0	+	0	0
A. ervi	7A, 7B, 7C	UK	3	*Hyperomyzus lactucae* (L.)		*Sonchus* sp.	+	+	+	0	+	0	0
A. funebris Mackauer	1A, 1B, 1C	wF	3	*Uroleucon* sp.	24	*Sonchus* sp.	+	+	+	+	+	+	+
A. funebris	2A, 2B, 2C	wF	3	Unknown		*Achillea* sp.	+	+	+	+	+	+	+
A. matricariae Haliday	1A, 1B, 1C	wF	3	*Hyalopterus pruni* (Geoffroy)	80	*Prunus spinosa*	+	+	+	+	+	+	+
A. matricariae	2A, 2B, 2C, 2D	wF	4	*Brachycaudus* sp.		*Matricaria* sp.	+	+	+	+	+	+	+
A. matricariae	3A, 3B	Chile, V	2	*Myzus persicae* (Sulzer)		*Nicotiana tabacum*	+	0	0	0	0	0	0
A. matricariae	4A, 4B	Chile, T	2	*Myzus persicae*		*Nicotiana tabacum*	+	0	0	0	0	0	0
A. matricariae	5A, 5B, 5C, 5D, 5E	wF	5	*Myzus persicae*		*Chenopodium* sp.	+	–	–	–	–	–	–
A. matricariae	6A, 6B, 6C	UK	3	*Myzus persicae*		*Chenopodium* sp.	+	–	–	0	–	0	0
A. matricariae	7A, 7B, 7C	UK	3	*Capitophorus carduinis* (Walker)		*Cirsium* sp.	+	–	–	0	–	0	0
A. microlophii Pennacchio & Tremblay	1A, 1B, 1C	wF	3	*Microlophium carnosum* (Buckton)	2	*Urtica dioica*	+	+	+	+	+	+	–
A. microlophii	2A, 2B, 2C	wF	3	*Microlophium carnosum*		*Urtica dioica*	+	+	+	+	+	+	–
A. rhopalosiphi De Stefani-Perez	1A, 1B, 1C	wF	3	*Sitobion avenae*	12	*Triticum aestivum*	+	+	+	–	+	+	+
A. rhopalosiphi	2A, 2B, 2C	eF	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	–	+	+	+
A. rhopalosiphi	3A, 3B, 3C, 3D	Chile, V	4	*Rhopalosiphum padi* (L.)		*Avena sativa*	+	0	0	0	0	0	0
A. rhopalosiphi	4A, 4B, 4C	Chile, V	3	*Schizaphis graminum* (Rondani)		Unknown	+	0	0	0	0	0	0
A. rhopalosiphi	5A, 5B, 5C	UK	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	0	+	0	0
A. rhopalosiphi	6A, 6B, 6C	UK	3	*Metopolophium dirhodum* (Walker)		*Triticum aestivum*	+	+	+	0	+	0	0
A. ribis Haliday	1A, 1B, 1C	wF	3	*Cryptomyzus ribis* (L.)	5	*Ribes rubrum*	+	X	+	+	+	+	+
A. ribis	2A, 2B, 2C	wF	3	*Cryptomyzus ribis*		*Ribes rubrum*	+	X	+	+	+	+	+
A. rosae Haliday	1A, 1B, 1C	wF	3	*Macrosiphum rosae* (L.)	4	*Rosa* sp.	+	+	+	–	+	+	+
A. rosae	2A, 2B, 2C	wF	3	*Macrosiphum rosae*		*Rosa* sp.	+	+	+	–	+	+	+
A. salicis Haliday	1A, 1B, 1C	wF	3	*Cavariella aegopodii* (Scopoli)	9	*Salix* sp.	+	+	+	–	+	+	–
A. schimitscheki Starý	1A, 1B, 1C	wF	3	*Elatobium abietinum* (Walker)	1	*Picea* sp.	+	+	+	+	+	+	+
A. sonchi Marshall	1A, 1B, 1C	wF	3	*Hyperomyzus lactucae*	4	*Sonchus* sp.	+	+	+	+	+	+	+
A. urticae Haliday	1A, 1B, 1C, 1D	wF	4	*Microlophium carnosum*	38	*Urtica dioica*	+	+	+	+	+	+	–
A. urticae	2A, 2B	wF	2	*Acyrthosiphum pisum*		*Vicia faba*	+	+	+	+	+	+	+
A. urticae	3A, 3B	eF	2	*Acyrthosiphum pisum*		*Trifolium* sp.	+	+	+	+	+	+	+
A. urticae	4A, 4B, 4C	UK	3	*Microlophium carnosum*		*Urtica dioica*	+	+	+	0	+	0	0
A. urticae	5A, 5B, 5C	UK	3	*Acyrthosiphum pisum*		*Pisum sativum*	+	+	+	0	+	0	0
A. uzbekistanicus Luzhetzki	1A, 1B, 1C	eF	3	*Sitobion avenae*	13	*Triticum aestivum*	+	+	+	+	+	+	+
D. rapae (M'Intosh)	1A, 1B, 1C	wF	3	*Hayhurstia atriplicis* (L.)	44	*Chenopodium album*	+	+	+	+	+	+	+
D. rapae	2A, 2B, 2C	wF	3	*Hayhurstia atriplicis*		*Chenopodium album*	+	+	+	+	+	+	+
D. rapae	3A, 3B, 3C	wF	3	*Hayhurstia atriplicis*		*Chenopodium album*	+	+	+	+	+	+	+
D. rapae	4A, 4B, 4C	wF	3	*Brevicoryne brassicae* L.		*Brassica* sp.	+	+	+	+	+	+	+
D. rapae	5A, 5B, 5C	wF	3	*Brevicoryne brassicae*		*Brassica* sp.	+	+	+	+	+	+	+
D. rapae	6A, 6B, 6C	wF	3	*Brevicoryne brassicae*		*Brassica* sp.	+	+	+	+	+	+	+
D. rapae	7A, 7B, 7C	UK	3	*Brevicoryne brassicae*		*Brassica* sp.	+	+	+	0	+	0	0
D. rapae	8A, 8B, 8C	UK	3	*Myzus persicae*		*Brassica* sp.	+	+	+	0	+	0	0

Table 1. continued

Species[a]	Voucher numbers	Location	No. of individuals	Aphid host	No. of hosts	Plant species	COI	CytB	16S	28S	LWRh	ArgK	EF
E. helleni Mackauer	1A, 1B, 1C	wF	3	*Cavariella* sp.	4	*Heracleum* sp.	+	+	+	+	+	+	–
E. lacertosus (Haliday)	1A, 1B, 1C	wF	3	*Microlophium carnosum*	12	*Urtica dioica*	+	+	+	+	+	–	+
E. nacheri Quilis-Perez	1A, 1B, 1C	wF	3	*Aphis fabae* Scopoli		*Chenopodium album*	+	–	+	+	+	+	–
E. nacheri	2A, 2B, 2C	wF	3	*Hayhurstia atriplicis*		*Chenopodium album*	+	–	+	+	+	+	–
E. nacheri	3A, 3B, 3C	UK	3	*Aphis fabae*		*Chenopodium album*	+	–	+	0	+	0	0
E. plagiator (Nees)	1A, 1B, 1C	wF	3	*Sitobion avenae*	104	*Triticum aestivum*	+	+	+	+	+	+	+
E. plagiator	2A, 2B, 2C	wF	3	*Macrosiphum rosae*		*Rosa* sp.	+	+	+	+	+	+	+
E. plagiator	3A, 3B, 3C	wF	3	*Aphis frangulae* Kaltenbach		*Frangula* sp.	+	+	+	+	+	+	+
E. plagiator	4A, 4B, 4C	UK	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	0	+	0	0
E. plagiator	5A, 5B, 5C	UK	3	*Metopolophium dirhodum*		*Triticum aestivum*	+	+	+	0	+	0	0
E. plagiator	6A, 6B. 6C	UK	3	*Aphis fabae*		*Rumex* sp.	+	+	+	0	+	0	0
L. cardui (Marshall)	1A, 1B, 1C	wF	3	*Aphis fabae*	9	*Chenopodium* sp.	+	–	+	+	–	–	+
L. cardui	2A, 2B, 2C	wF	3	*Aphis fabae*		*Cirsium arvense*	+	–	+	+	–	–	+
L. cardui	3A, 3B, 3C	wF	3	*Aphis rumicis* L.		*Rumex* sp.	+	–	+	+	–	–	+
L. cardui	4A, 4B, 4C	UK	3	*Aphis fabae*		*Cirsium* sp.	+	–	+	0	–	0	0
L. confusus Tremblay & Eady	1A, 1B, 1C	wF	3	*Aphis farinosa* Gmelin	23	*Salix* sp.	+	–	+	+	+	+	+
L. confusus	2A, 2B, 2C	wF	3	*Aphis rumicis*		*Salix* sp.	+	–	+	+	+	+	+
L. fabarum (Marshall)	1A, 1B, 1C	wF	3	*Aphis urticata* Gmelin	108	*Urtica dioica*	+	–	+	+	–	–	+
L. fabarum	2A, 2B, 2C, 2D, 2E, 2F	wF	6	*Aphis newtoni* Theobald		*Iris foetidissima*	+	–	+	+	–	–	+
L. fabarum	3A, 3B, 3C, 3D	wF	4	*Aphis hederae* Kaltenbach		*Hedea helix*	+	–	+	+	+	+	+
L. fabarum	4A, 4B, 4C	wF	3	*Aphis* sp.		Unknown	+	–	+	+	+	+	+
L. fabarum	5A, 5B, 5C	wF	3	*Aphis fabae*		*Cirsium arvense*	+	–	+	+	+	+	+
L. fabarum	6A, 6B, 6C	UK	3	*Aphis fabae*		*Cirsium arvense*	+	–	+	0	+	0	0
P. barbatum Mackauer	1A, 1B, 1C	wF	3	*Acyrthosiphon pisum*	1	*Medicago sativa*	+	+	+	+	+	–	+
P. barbatum	2A, 2B, 2C	eF	3	*Acyrthosiphon pisum*		*Medicago sativa*	+	+	+	+	+	–	+
P. barbatum	3A, 3B, 3C	UK	3	*Acyrthosiphon pisum*		*Trifolium* sp.	+	+	+	0	+	0	0
P. bicolor Mackauer	1A, 1B, 1C	wF	2	*Schizaphis* sp.	4	*Pinus* sp.	+	+	+	+	+	–	+
P. flavinode (Haliday)	1A, 1B, 1C	wF	3	*Tuberculatus annulatus* (Hartig)	24	*Quercus* sp.	+	+	+	+	+	–	+
P. flavinode	2A, 2B, 2C	wF	3	*Eucallipterus tiliae* L.		*Tiliae* sp.	+	+	+	+	+	–	+
P. volucre (Haliday)	1A, 1B, 1C	wF	3	*Acyrthosiphon pisum*	92	*Viciae* faba	+	+	+	+	+	–	+
P. volucre	2A, 2B, 2C	eF	3	*Unknown*		*Taraxacum* sp.	+	+	+	+	+	–	+
P. volucre	3A, 3B, 3C, 3D, 3E	wF	5	*Sitobion avenae*		*Triticum aestivum*	+	+	+	+	+	–	+
P. volucre	4A, 4B, 4C	eF	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	+	+	–	+
P. volucre	5A, 5B	wF	2	*Myzocallis coryli* (Goeze)		*Corylus avellana*	+	+	+	+	+	–	+
P. volucre	6A, 6B, 6C	wF	3	*Cavariella* sp.		*Heracleum* sp.	+	+	+	+	+	–	+
P. volucre	7A, 7B, 7C	wF	3	*Macrosiphum rosae*		*Rosa* sp.	+	+	+	+	+	–	+
P. volucre	8A, 8B, 8C	Belgium[b]	3	Unknown		Unknown	+	+	+	+	+	–	+
P. volucre	9A, 9B, 9C	UK	3	*Sitobion avenae*		*Triticum aestivum*	+	+	+	0	+	0	0
P. volucre	10A, 10B, 10C	UK	3	*Metopolophium dirhodum*		*Triticum aestivum*	+	+	+	0	+	0	0
P. yomenae Takada	1A, 1B, 1C	wF	3	*Uroleucon* sp.	14	*Centaurea jacea*	+	+	+	+	+	–	+
P. yomenae	2A, 1B, 1C	wF	3	*Uroleucon* sp.		Crepis *sp.*	+	+	+	+	+	–	+

COI, cytochrome *c* oxidase I; Cyt B, cytochrome B; 16S, rRNA 16S; 28S, rRNA 28S; LWRh, long wavelength rhodopsin; ArgK, arginine kinase; EF, elongation factor 1α.

[a]Genus abbreviations are as follows: *A.*, *Aphidius*; *D.*, *Diaeretiella*; *E.*, *Ephedrus*; *L*, *Lysiphlebus*; *P.*, *Praon.*

[b]Individuals originated from the breeding stock of Viridaxis S.A. (Gilly, Belgium).

Species name (based on morphological identification), specimen voucher numbers, sampling location [eF, east France; wF, west France; T, Temuco (Chile); V, Valdivia (Chile); UK, United Kingdom], number of individuals collected, host aphid of sampling, host range according to literature (see Appendix S1; # hosts), plant species where aphids were collected and gene fragments sequenced (+, successful sequencing; –, no DNA amplification; X, pseudogene/NUMT; 0, missing data). Each line of the table represents specimens from the same morphospecies, collected in the same location and reared from the same aphid species. Generalist morphospecies are in bold.

Cotesia flavipes (Hymenoptera: Microgastrinae) was added and used as outgroup. Parasitoids belonging to another subfamily of Braconidae (in particular from the genus *Cotesia*) have been classically used as outgroups in phylogenetic studies devoted to Aphidiinae (Smith *et al.*, 1999; Kambhampati *et al.*, 2000). The sequences of *C. flavipes* used were GQ853456 (COI), DQ459001 (CytB), DQ538530 (16S), DQ538977 (28S), DQ538703 (LWRH), DQ538925 (ArgK) and DQ538638 (EF).

To detect putative cryptic species, ML analyses were built for each gene fragment independently, while using all sequences obtained. First, we used the Akaike information criteria corrected for small sample sizes (Hurvich & Tsai, 1989) to select the best-fitting substitution models with JMODELTEST (Posada, 2008) for each gene. We then built ML trees for each gene using RAXML 7.2.7 (Stamatakis, 2006). Node support was assessed by bootstrapping (1000 pseudoreplicates). We then repeated this process and build an ML tree based on the combined seven genes using SEQUENCEMATRIX (Vaidya *et al.*, 2011); the data were partitioned into mitochondrial (COI and CytB), nuclear (ArgK, EF and LWRh) and ribosomal regions (16S and 28S).

Finally, we calculated pairwise intraspecific sequence divergences with MEGA version 5 (Tamura *et al.*, 2011) within each morphological group (substitution type, nucleotide; model, K2P). We consider here divergences between specimens from the same morphospecies as intraspecific divergences.

To find putative species boundaries on the ML trees, we performed bPTP using the web server (Zhang *et al.*, 2013) on the ML trees for each gene separately as well as on the ML tree of the seven genes. The convergence of MCMC chains was checked for each analysis. Posterior probabilities of species delimitation (PP) ≥ 0.90 from this analysis were considered as strong support.

Results

Parasitoid specimens reared mostly belonged to the seven generalist morphospecies, *A. ervi*, *A. matricariae*, *A. urticae*, *D. rapae*, *E. plagiator*, *L. fabarum* and *P. volucre*, and to the four specialist morphospecies, *Aphidius rhopalosiphi*, *Ephedrus nacheri*, *Lysiphlebus cardui* and *Praon barbatum*.

Both, the COI ML tree and the multilocus ML tree of the seven genes revealed that most of the generalist morphospecies have a substructure related to the aphid host (Figs 1, 2). Several generalist morphospecies were paraphyletic. The bPTP model delimited at least two distinct species in the generalist morphospecies, *A. matricariae* and *A.urticae*. In other cases, the bPTP model found support that specimens from different morphospecies may belong to the same species.

Ability of each gene fragment to reveal substructures within morphospecies

For all individuals, we amplified and successfully sequenced the COI fragment. The global success of amplification was 77.9% for CytB, 95% for 16S, 96.5% for 28S, 89.4% for LWRh, 66.7% for ArgK and 86.4% for EF (Table 1). For *A. matricariae*, we were unable to amplify specimens emerged from *Myzus persicae* for any gene except COI, while we successfully amplified all other specimens of *A. matricariae* emerged from other aphid hosts. For *L. fabarum*, we were unable to amplify specimens reared from *Aphis urticata* and *Aphis newtoni* for LWRh and ArgK, while we successfully amplified the other specimens coming from other aphid hosts.

Mitochondrial genes, especially COI, delimited the highest number of species with the bPTP model [12 species delimited with PP ≥ 0.90 with COI (Fig. 1); seven with CytB (Fig. S1)]. Moreover, COI revealed substructures in most of the generalist morphospecies (except *A. ervi*) and in several specialist species (*A. rhopalosiphi*, *Aphidius ribis*, *P. barbatum*; Fig. 1, Table 2). The 16S rRNA gene and the nuclear genes (LWRh, EF) were inefficient to delimit species with bPTP models but nevertheless displayed substructure in several generalist morphspecies (Figs S2–S4). ArgK and 28S genes were inefficient to display substructure within the generalist morphospecies and to delimit species (Figs S5 and S6).

The clades displayed from the COI ML tree and the CytB ML tree were mostly consistent. However, unlike the COI ML tree, we did not find substructure within *D. rapae* specimens with the CytB ML tree. Moreover, in the CytB ML tree, *E. plagiator* was split into three groups instead of two in the COI ML tree (Figs 1, S1).

The 16S and LWRh ML trees revealed very little substructure within the morphospecies (Figs S2 and S3). The substructure of *P. volucre* in the 16S and LWRh ML trees was different from that in the COI ML tree. In the 16S ML tree, *P. volucre* specimens from *Myzocallis coryli* (5A, 5B) were separated from specimens of *P. volucre* found on *Cavariella* sp., *Macrosiphum rosae* and on an unknown aphid host coming from Belgium (6A, 6B, 6C, 7A, 7B, 7C, 8A, 8B, 8C; Figure S2). In the LWRh tree, specimens from Belgium (8A, 8B, 8C) were separated from the remaining *P. volucre* specimens. However, all specimens (5A, 5B, 6A, 6B, 6C, 7A, 7B, 7C, 8A, 8B, 8C) had the same COI sequence (Fig. 1).

The EF ML tree displayed substructure only for *E. plagiator*, *P. volucre* and *L. fabarum* (Fig. S4). However, *P. volucre* and *L. fabarum* were separated in more clades in the COI ML tree.

Identification of substructure within morphospecies

The ML analysis and the bPTP models based on COI and on the seven genes together revealed that *A. urticae* and *A. matricariae* contain several clades (Figs 1, 2, S7). The distance between individuals from the same morphospecies was the highest observed in *A. matricariae* (with COI) and in *A. urticae* (with all genes, Table 2). *Aphidius matricariae* was separated in four paraphyletic clades according mainly to the aphid host species. Moreover, *A. matricariae* specimens reared on *M. persicae* are separated into two clades: one includes specimens from France and UK, and the other includes specimens from Chile (Figs 2, S7). The bPTP model revealed that several *A.*

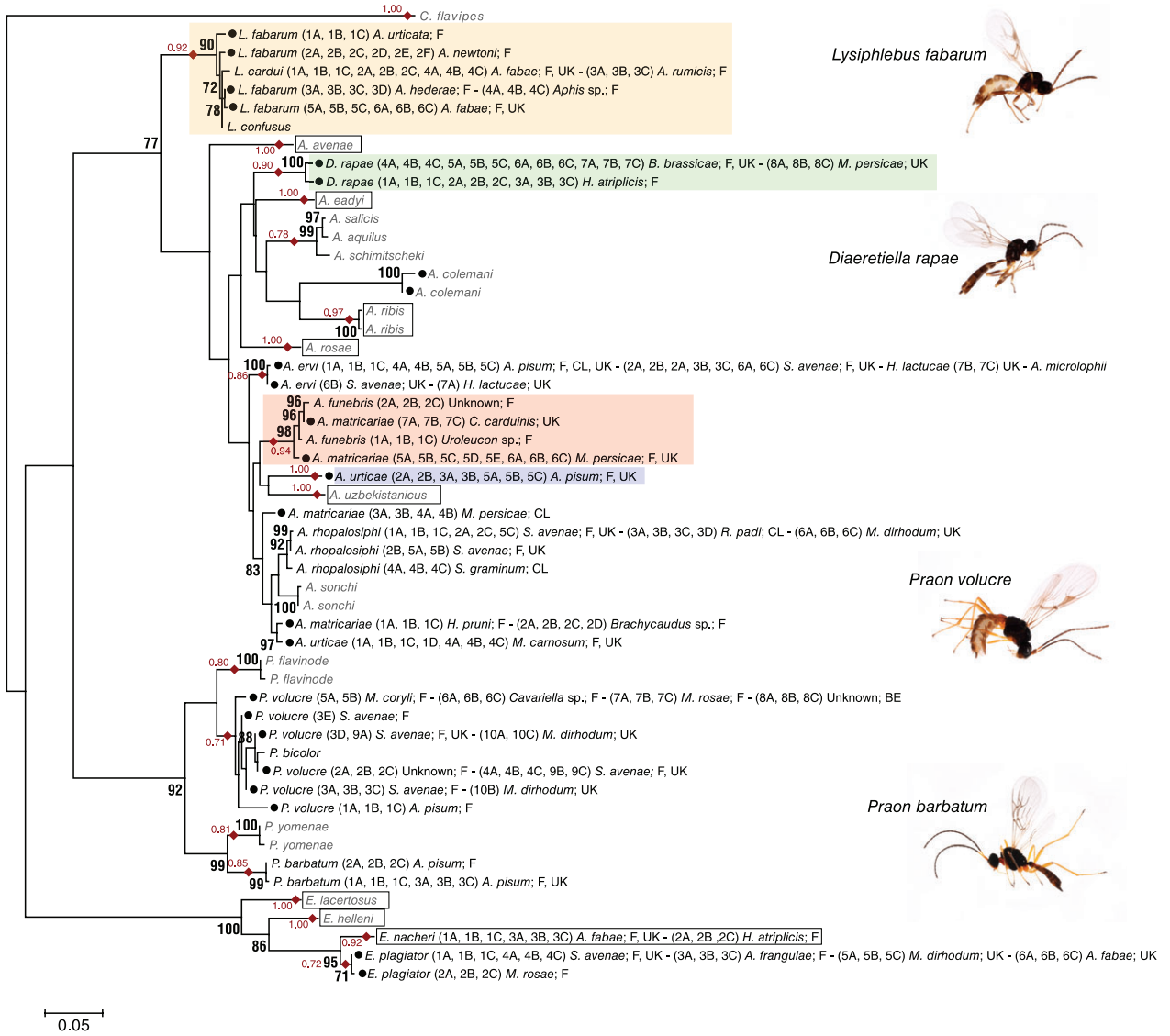

Fig. 1. Maximum likelihood tree obtained from cytochrome *c* oxidase I fragment. Information is presented in the following order: parasitoid morphospecies name; specimen voucher numbers (in brackets; see Table 1); aphid host and sampling location. Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the Bayesian Poisson tree processes (bPTP) model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Black frames indicate bPTP-suggested species involving only specialists; colours indicate bPTP-suggested species involving generalist morphospecies (*Aphidius matricarie* in red, *Aphidius urticae* in blue, *Diaeretiella rapae* in green and *Lysiphlebus fabarum* in yellow). Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

matricariae specimens (5A, 5B, 5C, 5D, 5E, 6A, 6B, 6C, 7A, 7B, 7C) and all *Aphidius funebris* specimens belong to the same species (Fig. 2). *Aphidius urticae* was separated in two paraphyletic clades: one with specimens reared on *Microlophium carnosum*, and one with specimens reared on *Acyrthosiphon pisum*.

Other generalist morphospecies were found to be paraphyletic as well: *L. fabarum* (Figs 1, 2, S8) and *P. volucre* (Figs 1, 2, S9); the intraspecific distances were, however, smaller than distances calculated for *A. urticae* and *A. matricariae*. Specimens from *L. fabarum* were separated in four paraphyletic clades according to the aphid host species (Figs 2, S8). However, the bPTP revealed that all specimens from the genus *Lysiphlebus* belonged to the same species (PP = 0.98; Fig. 2). For *P. volucre,* the genetic divergence observed depended mainly on the aphid host species; however, species emerged from the aphid host *Sitobion avenae* were separated in several clades (Figs 2, S9). *Praon bicolor* was nested in the clade formed by all *P. volucre* specimens and they were considered a single species with bPTP (PP = 0.95; Figs 2, S9).

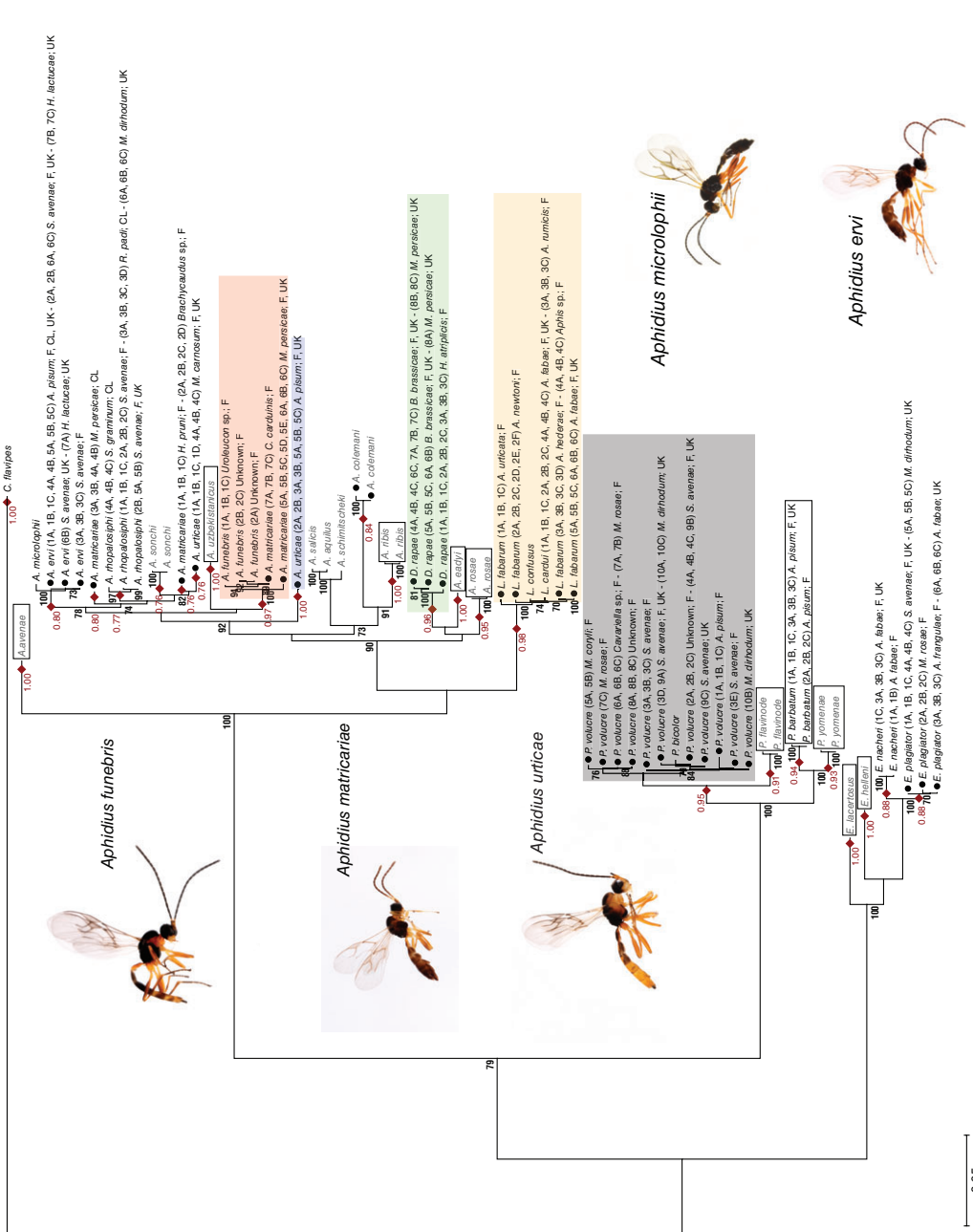

Fig. 2. Maximum likelihood tree obtained from the multilocus analysis based on seven genes. Information is presented in the following order: parasitoid morphospecies name; specimen voucher numbers (in brackets; see Table 1); aphid host and sampling location. Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the Bayesian Poisson tree processes (bPTP) model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Black frames indicate bPTP-suggested species involving only specialists; colours indicate bPTP-suggested species involving generalist morphospecies (*Aphidius matricarie* in red, *Aphidius urticae* in blue, *Diaeretiella rapae* in green, *Lysiphlebus fabarum* in yellow and *Praon volucre* in grey). Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Table 2. Maximum and mean intraspecific distances between two samples for each parasitoid morphospecies.

	COI		CytB		16S		28S		LWRh		ArgK		EF	
Species	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Aphidius ervi	0	0	0	0	0	0	0	0	0	0	0.002	0.002	0	0
Aphidius matricariae	0.05	0.027	0.05	0	0	0	0	0	0	0	0	0	0	0
Aphidius rhopalosiphi	0.007	0.002	0.007	0	0	0	0	0	0	0	0	0	0	0
Aphidius urticae	0.06	0.032	0.058	0.03	0.016	0.009	0.039	0.022	0.026	0.014	0.016	0.009	0	0
Diaeretiella rapae	0.01	0.005		0	0	0	0	0	0.015	0.007	0	0	0	0
Ephedrus nacheri	0	0	NA	NA	0	0	0	0	0.002	0.001	0	0	NA	NA
Ephedrus plagiator	0.002	0.001	0.005	0	0	0	0	0	0.004	0.002	0	0	0.002	0.001
Lysiphlebus cardui	0	0	NA	NA	0	0	0	0	NA	NA	NA	NA	0	0
Lysiphlebus fabarum	0.013	0.006	NA	NA	0.003	0.001	0	0	0	0	0	0	0.002	0.001
Praon barbatum	0.002	0.001	0.002	0	0	0	0	0	0	0	NA	NA	0	0
Praon volucre	0.018	0.013	0.014	0.01	0.016	0.006	0	0	0.003	0.001	NA	NA	0.002	0.001

COI, cytochrome *c* oxidase I; Cyt B, cytochrome B; 16S, rRNA 16S; 28S, rRNA 28S; LWRh, long wavelength rhodopsin; ArgK, arginine kinase; EF, elongation factor 1α.

The specialist *A. rhopalosiphi* and the generalists *D. rapae* and *E. plagiator* appear to comprise several closely related subspecies, but they remain monophyletic (Figs 1, 2, S7, S10). For *A. rhopalosiphi*, Chilean specimens coming from *Schizaphis graminum* were separated from the remaining specimens (Figs 2, S7). *Diaeretiella rapae* was separated in two clades belonging to the same species according to the bPTP model (PP = 0.96): a clade with specimens emerged from *Hayhurstia atriplicis* and a clade with specimens emerged from *Brevicoryne brassicae* and *M. persicae* (Figs 2, S7). For *E. plagiator*, specimens from *M. rosae* were separated from the remaining *E. plagiator* specimens (Figs 2, S10).

By comparison, the generalist *A. ervi* and the specialists *E. nacheri*, *L. cardui* and *P. barbatum* exhibited no or very low variation in sequence unrelated to the host species irrespective of which gene fragment was analysed (Figs 1, 2).

Discussion

Using multigene molecular barcoding revealed either molecular substructure or existence of cryptic species related to aphid host in most of the Aphidiinae morphospecies considered until now as generalists, probably documenting cryptic specialization. The species delimitation method used (Zhang *et al.*, 2013) revealed several cryptic species in some generalist morphospecies. However, this approach also grouped specimens from different morphospecies into the same species, thus demonstrating mismatches between morphological and molecular identification that will require further investigation.

The COI fragment was the most polymorphic, allowing us to distinguish groups that did not differ in their sequences with other genes. As expected, the rRNA 28S fragment was the least polymorphic marker and showed no sequence variation in several species (*D. rapae*, *E. plagiator*, *L. fabarum* and *P. volucre*). However, despite a globally lower level of variability when compared with mitochondrial fragments, ribosomal and nuclear genes appeared to provide supplementary information within several species (*A. ervi*, *E. plagiator* and *P. volucre*) as well as a better support in the species delimitation analysis. Consequently, we consider the complementary use of seven genes the most reliable approach to delimit species in the Aphidiinae using the bPTP model. Our study confirmed the conclusions of Derocles *et al.* (2012), supporting the use of multiple genes (mitochondrial, ribosomal and nuclear) to inform accurate identifications of cryptic species in the Aphidiinae.

For *A. matricariae* and *A. urticae*, our study not only revealed a genetic structure related to host specialization, but also found paraphyly with respect to other species in the *Aphidius* genus. The case of *A. matricariae* was complex. The sampled individuals appeared strongly separated in four paraphyletic subgroups. The first subgroup contained French specimens sampled on *Brachycaudus* sp. and *Hyalopterus pruni*. The second subgroup included individuals from Chile (Valdivia and Temuco) on *M. persica*e. The third subgroup was composed of specimens from France and the UK sampled on *M. persicae*. Finally, the last subgroup was composed of specimens emerged from

Capitophorus carduinis. Hence, the intraspecific differentiation in the morphospecies *A. matricariae* appeared to result from both host specialization and geographical differentiation. To clarify the status of this morphospecies, more comprehensive sampling of a larger number of host species from a wider range of geographical locations will be required. The paraphyletic grades observed for *A. matricariae* and *A. urticae* were rather surprising. Contaminations during PCR amplifications and sequencing are unlikely to explain this result, as these individuals were not all processed and sequenced together. Such genetic divergences could also result from misidentification rather than true biological divergence. Fortunately, thanks to the protocol used for DNA extraction, it was possible to re-examine all individuals. Initial identifications based on morphological characters were confirmed both by a new morphological examination and by comparing the sequences obtained to public databases (i.e. GenBank). Despite the diagnostic characters examined (maxillary palps, ovipositor sheats, number of antennal segments, wing size, shape and veins, anterolateral area of petioles, commonly used for Aphidiinae identification; Kavallieratos *et al.*, 2001, 2005; Tomanović *et al.*, 2003, 2007), no morphological character could be found to distinguish individuals belonging to different genetic clades, which is generally the case when cryptic species are revealed by molecular analyses (Hebert *et al.*, 2004; Smith *et al.*, 2007: 2208). Consequently, species delimitation remains based on solely on the COI fragment for *A. matricariae* and should be tested using additional genes (nuclear genes and rDNA). Further investigations on both *A. matricariae* and *A. urticae* should also focus on the identification of potential diagnostic morphological traits.

The results for *L. fabarum*, *P. volucre*, *E. plagiator* and *D. rapae* were consistent with the literature: previous studies showed cryptic species and host specialization or at least host preference for these four generalists (Tomić *et al.*, 2005; Antolin *et al.*, 2006; Stilmant *et al.,* 2008; Barahoei *et al.*, 2011; Le Ralec *et al.*, 2011). However, it should be noted that we cannot directly compare our study with the literature because we examined different aphid host species. Our results nevertheless showed substructure associated with the aphid host for these four 'generalist' species. Moreover, as observed in *A. matricariae* and *A. urticae*, *L. fabarum* and *P. volucre* did not form a monophyletic group. Indeed, their sister species (*Lysiphlebus confusus* and *P. bicolor*, respectively) appeared nested within the clade constituted by *L. fabarum* and *P. volucre* specimens.

Finally, *A. ervi* is the only species in which sequence variation consistent with host species was absent. This may result from the fact that only three host species were sampled. Moreover, molecular tools used here may not be powerful enough to distinguish potential cryptic species within *A. ervi*. For example, *A. ervi* and its sister species *Aphidius microlophii* (split based on ecological and morphological criteria by Pennacchio & Tremblay, 1986) are difficult to discriminate based on molecular data (Derocles *et al.*, 2012), even when several genes are analysed. Alternatively, *A. ervi* could be the only 'true generalist' present in this study.

Our study needs to be extended in terms of the number of sampled populations, for both generalists and specialists. A complete analysis would require exhaustive sampling of all host aphids in several locations to assess the relative contribution of geography and host specialization to genetic differentiation. Previous studies have shown that the number of potential hosts is high in some Aphidiinae species and several aphid–parasitoid interactions are rarely observed in nature (e.g. more than 70 cited host species for *L. fabarum* and *P. volucre*; Kavallieratos *et al.*, 2004; Starý, 2006). Moreover, other Aphidiinae species need to be considered in future studies. *Lysiphlebus testaceipes* (Cresson), in particular, is another extremely polyphagous species (almost 200 hosts), introduced in Europe and currently suspected to become an invasive species (Žikić *et al.*, 2015).

Whether the genetic substructure revealed by our study within several generalist morphospecies represents intraspecific variation or reproductively isolated cryptic species will also require further work. However, the ecological separation of genetic clades revealed here does suggest that they represent important biological units whatever their final nomenclature. Indeed, all specimens from the genus *Lysiphlebus* were grouped in a single species based on the bPTP model. Similarly, *A. funebris* was grouped with several *A. matricariae* specimens in the same species as well as *P.volucre* with *P.bicolor* (and *A. ervi* with *A. microlophii*, even though this was not well supported by the bPTP model). This result was rather surprising, as the following have distinctive morphological characters: *L. cardui*, *L. confusus* and *L. fabarum* (Petrović *et al.*, 2015); *A. matricariae* and *A. funebris* (Tomanović *et al.*, 2003); *P. bicolor* and *P.volucre* (Kavallieratos *et al.*, 2005).

As demonstrated recently (Kekkonen & Hebert, 2014), a combination of several analytic methods, such as the General Mixed Yule-coalescent (GMYC; Pons *et al.*, 2006), the Automatic Barcode Gap Discovery (Puillandre *et al.*, 2012) and the Barcode Index Number system (Ratnasingham & Hebert, 2013), could provide a better support to species delimitation. A previous study on Aphidiinae molecular identification (Derocles *et al.*, 2012) nevertheless demonstrated that GMYC analysis does not provide a satisfactory species delimitation method in this subfamily. Indeed, all species with specimens exhibiting genetic divergences (even if very low or based on a single nucleotide change) were systematically split into different independent evolutionary entities in the GMYC analysis, thus showing many disagreements with both morphology and the bPTP model used in the present study. From our point of view, this demonstrates that molecular markers cannot always be used alone to delimit species, but instead should be complemented with ecological and morphological data (Tan *et al.*, 2009). Genetically described cryptic species nevertheless provide a framework for more exhaustive morphological examination of the specimens to develop diagnostic characters (Baur *et al.*, 2014; Al Khatib *et al.*, 2014). Such an integrative taxonomic approach will be very powerful in disentangling the cryptic and functional diversity among Aphidiinae if combined with laboratory experiments, such as crossing between putative cryptic species or host acceptance, to confirm the host specialization suggested here (for aphid species that can be reared in the laboratory).

The general theme from our studies is the presence of genetic differentiation related to host specialization and/or geography in most species considered until now as generalist Aphidinae. While genetic differentiation also occurred in the specialists, the distances between specimens in specialists were lower than in the generalist morphospecies, and specialists were never splitted into several species by the bPTP model. This is an important finding that changes our view of the structure of species-interaction networks between aphid and parasitoid communities (Derocles *et al.*, 2014). The identification of cryptic species with molecular approaches renews our vision of trophic networks (Kaartinen *et al.*, 2010) and can drastically change the inferred topology of the ecological network studied (Wirta *et al.*, 2014). From an evolutionary point of view, our findings also suggest that the generalist strategy is rarely maintained in aphid–parasitoid interactions and that it is highly unstable, with a tendency for generalists to differentiate into host specialized species. Finally, from an applied point of view, this host specialization may have important consequences on the potential use of generalist morphospecies as biocontrol agents against agricultural pests (de Conti *et al.*, 2008). In a context of augmentation biocontrol, a generalist morphospecies including several specialist species, reared on a single aphid host species, could fail to parasitize other target aphids in fields. Similarly, for conservative biocontrol, nonpest aphids in seminatural habitats could not be a pool of biocontrol agents against pest aphids.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12160

DNA sequences were assigned GenBank accessions: KP982920-KP984456. Sequence alignments and trees: Dryad: doi:10.5061/dryad.qm1v0

Figure S1. ML tree obtained from CytB fragment. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S2. ML tree obtained from 16S fragment. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S3. ML tree obtained from LWRh fragment. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S4. ML tree obtained from EF fragment. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S5. ML tree obtained from ArgK fragment. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S6. ML tree obtained from 28S fragment. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S7. ML tree of *Aphidius* genus obtained from the multilocus analysis based on seven genes. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posteriorior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa

discussed in detail in the results are in black, and other taxa are in grey.

Figure S8. ML tree of *Lysiphlebus* genus obtained from the multilocus analysis based on seven genes. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posteriorior delimitation probabilities ≥ 0.70. Poster delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S9. ML tree of *Praon* genus obtained from the multilocus analysis based on seven genes. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Figure S10. ML tree of *Ephedrus* genus obtained from the multilocus analysis based on seven genes. Specimen voucher numbers are in brackets (see Table 1). Bootstrap values (≥ 70; in black) are given for each branch. Scaling is expressed in the proportion of substituted bases per site. Red diamonds indicate species delimited with the bPTP model with posterior delimitation probabilities ≥ 0.70. Posterior delimitation probabilities (≥ 0.70) are displayed in red. Generalist species are marked with a black circle. Taxa discussed in detail in the results are in black, and other taxa are in grey.

Table S1. Genes, primers, hybridization temperatures and size of the amplified fragments used in this study.

Appendix S1. References used to define Aphidiinae parasitoid host range in Table 1.

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