

# First Nearctic record of *Diolcogaster claritibia* (Hymenoptera: Braconidae: Microgastrinae), with notes on taxonomic status and natural history

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**Abstract**—The Microgastrinae wasp *Diolcogaster claritibia* (Papp, 1959) (Hymenoptera: Braconidae), a parasitoid of the diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is recorded from the Nearctic region (Alberta, Manitoba, and Ontario, Canada) for the first time. In spite of some minor morphological and molecular (DNA barcoding) differences, the available data indicate that the European and New World specimens should be considered one species, but more specimens and loci need to be sampled to conclusively determine the phylogeographic history of the species. *Diolcogaster claritibia* seems to be widely distributed within the Holarctic, and the molecular data suggest that it was recently introduced to North America from Europe.

**Résumé**—Nous signalons pour la première fois la présence de la guêpe *Diolcogaster claritibia* (Papp, 1959) (Hymenoptera: Braconidae: Microgastrinae), un parasitoïde de la fausse-teigne des crucifères, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera Plutellidae), dans la région néarctique (Alberta, Manitoba et Ontario, Canada). Malgré de faibles différences morphologiques et moléculaires (codes-barres d'ADN), les données actuelles indiquent que les spécimens d'Europe et du Nouveau-Monde forment une même espèce; il faudrait cependant obtenir plus de spécimens et échantillonner plus de locus afin de déterminer avec plus de certitude l'histoire phylogéographique de l'espèce. *Diolcogaster claritibia* semble être réparti sur une grande échelle dans la région holarctique et les données moléculaires laissent croire qu'il a été introduit récemment en Amérique du Nord à partir de l'Europe.

## Introduction

*Diolcogaster claritibia* (Papp, 1959) (Hymenoptera: Braconidae: Microgastrinae) was reared from diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), one of the most damaging insect pests attacking cruciferous crops throughout its nearly cosmopolitan range. *Diolcogaster claritibia* has been reported only from the Palaearctic region, where it has been recorded from a number of countries.

Recent work on DNA barcoding of the world species of Microgastrinae has generated over 20 000 sequences (Smith *et al.* 2013), which has allowed for comparisons of material collected in

different biogeographic regions. Unidentified specimens whose DNA sequences match identified species have been studied further, and as a result many species previously unknown from a region are being recognised.

Here, we report for the first time the presence of *D. claritibia* in the Nearctic region, compare its populations with those of the Palaearctic region, and discuss the taxonomic status of the species.

## Methods

We performed a morphological examination of seven females from Europe (one from Cyprus, three from East Jordan, two from France, and one

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from the Netherlands), three males (one from Italy, two from Jordan), and close to 100 specimens from Canada (Alberta, Manitoba, and Ontario). Voucher specimens are deposited in the Canadian National Collection of Insects, Ottawa, Canada, and the National Museums of Scotland, Edinburgh, United Kingdom.

Morphological terms and measurements of structures are according to Mason (1981), Huber and Sharkey (1993), and Whitfield (1997). In the morphological section of the paper we use T1 and T2 to refer to mediotergites 1 and 2, respectively.

For molecular analyses, all *Diolcogaster* Ashmead, 1900 DNA barcodes (658 base pair region near the 5' terminus of the COI gene) available in the Barcode of Life Data System (BOLD) (<http://www.boldsystems.org>) were imported into Geneious Pro 6.1 (Drummond *et al.* 2011) along with one newly generated sequence of *D. claritibia* from the Netherlands. This new sequence was obtained following standard protocols for DNA extraction (Ivanova *et al.* 2006) and amplification using primers LepF1-LepR1 (Smith *et al.* 2006, 2007, 2008). Collection information and accessions (BOLD and GenBank) are available in Table 1. Fourteen of the 115 sequences were < 500 base pairs long and were therefore deleted from the data set before further analysis. The remaining 101 sequences (representing eight *Diolcogaster* species) were aligned using the default settings for MUSCLE (Drummond *et al.* 2011). The final aligned data set contained 657 characters.

To determine if the Canadian *Diolcogaster* specimens are conspecific with European *D. claritibia*, a neighbour-joining tree (Saitou and Nei 1987) was constructed in Geneious Pro 6.1 (Drummond *et al.* 2011) using the TN93 model (Tamura and Nei 1993) (Fig. 1). To further investigate this, a Bayesian phylogenetic analysis was also performed. The aligned data set was partitioned into two parts, the first containing first and second codon positions and the second containing third codon positions because the mutation rate of third codon positions is much higher than that of first and second codon positions. Model testing done in JModelTest v.2.2.2 (Darriba *et al.* 2012) using the Bayesian Information Criterion selected the GTR+I model for the first partition and the GTR+G model for the second partition. Two independent Bayesian analyses with four chains each were run in MrBayes v.3.2.1 (Ronquist and

Huelsenbeck 2003) for 92 million generations each. Trace files of all parameters were examined in Tracer v.1.5 (Rambaut and Drummond 2009) to verify that the runs had converged on the same stationary distribution, and to select the percentage of samples to remove as burn-in. A 10% burn-in was removed from both tree files that were then combined and resampled at 10%. A maximum clade credibility tree (MCCT) was made from the combined resampled tree file in TreeAnnotator v.1.7.5 (Rambaut and Drummond 2013).

Given that few sites were variable among the Canadian and European *D. claritibia* sequences, a haplotype network analysis was also constructed to see if the Canadian and European *D. claritibia* specimens represent different mitochondrial lineages. The network building software TCS (Clement *et al.* 2000), which uses coalescent theory (Hudson 1990; Templeton *et al.* 1992) and an iterative Bayesian method to evaluate the probability that DNA sequences share a parsimonious relationship without multiple substitutions underlying any single nucleotide difference, was used to construct a haplotype network of the *D. claritibia* DNA barcode sequences under a probability of parsimony of 0.95 and 0.93. Ambiguities were treated as missing data and all missing data was ignored in the analysis.

## Results

Specimens of *D. claritibia* were independently found by M.R.S. (from Italy and the Netherlands) and J.F.T. (from Canada) while identifying samples of Braconidae wasps. All are new country records for the species.

The Canadian specimens represent the first record for the Nearctic region and expand the known distribution of *D. claritibia* to the New World. Based on previous data, the species was known to be widely distributed across the Palearctic region from Spain to Kazakhstan and Russia (Chita and Krasnodar) (Yu *et al.* 2012).

Previously, *D. claritibia* had been reared from *P. xylostella* on *Sinapis alba* Linnaeus (Brassicaceae) (white mustard) and also collected as adults on *Lepidium* Linnaeus (Brassicaceae) species (Papp 1981). Here we record the species from *P. xylostella* (probably) on an undetermined large straggly crucifer in the Italian Alps, from *P. xylostella* on *Brassica oleracea* Linnaeus

**Table 1.** Details of specimens examined.

DNA voucher codes	GenBank accession	Haplotype #	Collecting date	Country /locality	Latitude	Longitude
CNCHYM 45357	KC130423	HT5	2012	The Netherlands, Wageningen		
			11.vi.2004	Italy, Burgeis, 1200 m		
CNCH1126		HT8	05.vi.1995	France, Bel Air	43.64	3.75
CNCH1127	KC130380	HT9	03.vi.1995	France, Baillargues	43.66	4.01
CNCH1132	JQ852198	HT1	29.vi.2003	Canada, Alberta, Lethbridge	49.75	-112.85
CNCHYM 00892	JQ851690	HT9	21.iv.1966	Cyprus, Amathus		
CNCHYM 00893			09.ii.1968	Jordan, Wadi Schaib		
CNCHYM 01690	JQ850581	HT1	23.vi.2010	Canada, Ontario, Ottawa, Central Experimental Farm		
CNCHYM 01691	JQ854254	HT1	23.vi.2010	Canada, Ontario, Ottawa, Central Experimental Farm		
CNCHYM 01692	JQ854023	HT1	23.vi.2010	Canada, Ontario, Ottawa, Central Experimental Farm		
CNCHYM 01693	JQ851862	HT1	23.vi.2010	Canada, Ontario, Ottawa, Central Experimental Farm		
CNCHYM 01694	JQ846915	HT1	23.vi.2010	Canada, Ontario, Ottawa, Central Experimental Farm		
CNCHYM 01695	JQ853552	HT1	23.vi.2010	Canada, Ontario, Ottawa, Central Experimental Farm		
HYM00000354		HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000355	JQ852035	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000356	JQ849975	HT4	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000357	JQ850145	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000358	JQ853195	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000359	JQ855427	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000361	JQ847237	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000363	JQ852605	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000364	JQ847704	HT1	25.v.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000365	JQ851624	HT1	17.vii.2006	Canada, Alberta, Lethbridge	49.72	-112.86
HYM00000436	JQ851002	HT1	22.vi.2006	Canada, Alberta, Stirling	49.50	-112.52
HYM00000437	JQ846796	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00000439	JQ848890	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00000441	JQ851276	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00000444	JQ852792	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00000447	JQ852995	HT1	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00000448	JQ851614	HT1	06.vi.2006	Canada, Alberta, Lethbridge	49.71	-112.85
HYM00000449	JQ847260	HT1	06.vi.2006	Canada, Alberta, Lethbridge	49.71	-112.85
HYM00000450	JQ850339	HT1	06.vi.2006	Canada, Alberta, Lethbridge	49.71	-112.85
HYM00001293	JQ852955	HT1	12.vi.2006	Canada, Manitoba, 10 km W of Morden	49.20	-98.25

Table 1. *Continued*

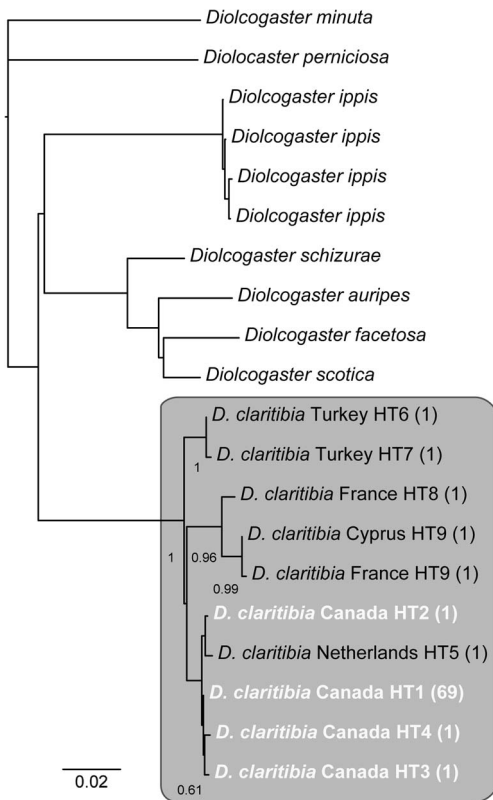
DNA voucher codes	GenBank accession	Haplotype #	Collecting date	Country /locality	Latitude	Longitude
HYM00001294	JQ847337	HT1	12.vi.2006	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001295	JQ854496	HT1	12.vi.2006	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001296	JQ847625	HT1	12.vi.2006	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001297	JQ847296	HT1	12.vi.2006	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001298	JQ854754	HT1	12.vi.2006	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001299	JQ846747	HT1	08.vi.2007	Canada, Manitoba, Spruce Woods Provincial Park	49.73	-99.29
HYM00001300	JQ849947	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001301	JQ849529	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001302	JQ852405	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001303	JQ852960	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001304	JQ854075	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001305	JQ852779	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001306	JQ851225	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001307	JQ848067	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001308	JQ847893	HT1	12.vi.2007	Canada, Manitoba, 10 km W of Morden	49.20	-98.25
HYM00001495	JQ847484	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001496	JQ848061	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001497	JQ847615	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001498	JQ854034	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001499	JQ852355	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001501	JQ853044	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001502	JQ847768		01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001503	JQ853752	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001504	JQ855204	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001505	JQ849102	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001507	JQ848660	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001508	JQ850635	HT1	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001510	JQ849757	HT1	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001512	JQ852728	HT1	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001513	JQ846601	HT1	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001514	JQ851259	HT2	06.vi.2006	Canada, Alberta, Lethbridge	49.71	-112.85
HYM00001515	JQ852317	HT1	06.vi.2006	Canada, Alberta, Lethbridge	49.71	-112.85
HYM00001516	JQ846627	HT3	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83

Table 1. Continued

DNA voucher codes	GenBank accession	Haplotype #	Collecting date	Country /locality	Latitude	Longitude
HYM00001517	JQ849860	HT1	07.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001518	JQ849587	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001519	JQ851977	HT1	06.vi.2006	Canada, Alberta, Lethbridge	49.71	-112.85
HYM00001520	JQ855421	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001521	JQ851590	HT1	27.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001522	JQ854200	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001523	JQ855217	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001524	JQ854884	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001526	JQ847448	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001527	JQ848617	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001528	JQ846790	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001529	JQ846754	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001530	JQ851322	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001531	JQ851465	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001532	JQ848586	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001533	KC130427	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001534	JQ850098	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001535	JQ851250	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83
HYM00001536	JQ848008	HT1	01.vi.2006	Canada, Alberta, Lethbridge	49.69	-112.83

The column "Haplotype #" refers to numbers of different haplotypes as shown in Fig. 2.

**Fig. 1.** Neighbour-joining tree of COI DNA barcodes of *Diolcogaster* species. One representative of each haplotype (HT) is included (except for *D. claritibia* haplotype 2, which shows specimens found in both Cyprus and France). The number of specimens within each *D. claritibia* haplotype is indicated within parentheses. Posterior probabilities above 0.50 for *D. claritibia* groups recovered in the Bayesian analysis are shown below branches.



(cabbage) in the Netherlands, and from *P. xylostella* on *Brassica juncea* (Linnaeus) Czernajew, *B. napus* Linnaeus (canola), and *S. alba* (Brassicaceae) in Canada (agricultural areas of Alberta, Manitoba, and Ontario).

Based on previous references (compiled in Yu *et al.* 2012) 31 species of Microgasterinae are recorded as parasitoids of *P. xylostella* worldwide – though many of those records are likely to be wrong (*sensu* Shaw 1994). Most belong to *Apanteles* Forster, 1862 and *Cotesia* Cameron, 1891, with *D. claritibia* being the only species of *Diolcogaster* recorded from diamondback moth.

### Morphological studies on *Diolcogaster claritibia*

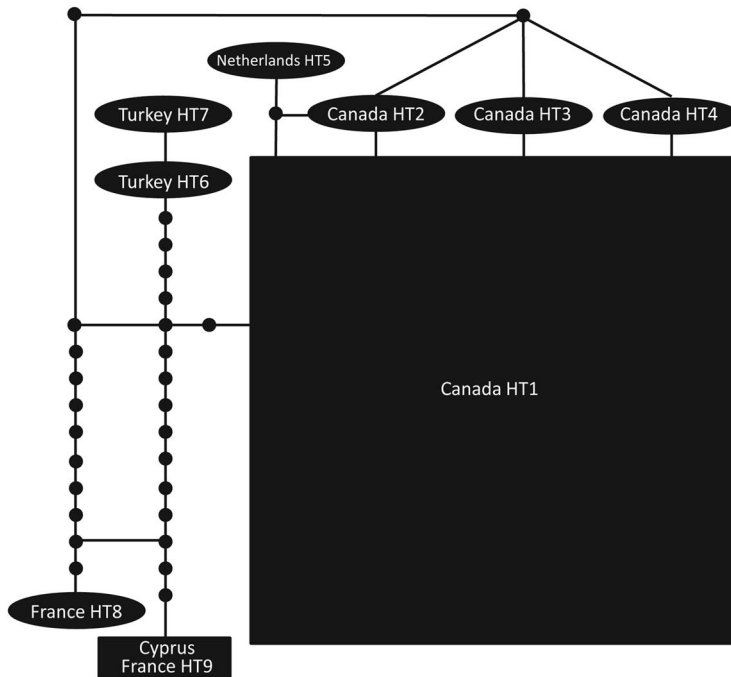
**Species description.** (Figs 3–4). Body length: 2.0–2.2 mm. Fore wing length 2.0–2.4 mm. Prothorax fully yellow, mesothorax and metathorax black on apical half, yellow on basal half, rest of body fully black. Mesocutum weakly punctate with very shallow punctures. Propodeum with relatively weakly sculpture, with some rugosity around the median carina, the rest mostly smooth. T1 slightly widening up to 0.7 of tergite length (where widest point occurs), then narrowing towards posterior margin (width at posterior margin slightly wider than width at anterior margin). T1 1.3 times as long as its width at posterior margin. T1 length/maximum width 1.5–1.6 times in European specimens and 1.2–1.6 times in Canadian specimens. T2 fully sculptured at least posteriorly, with the elevated median field a little more strongly sculptured than the side areas, and each side area divided into a sculptured and almost rhomboidal posterior area slightly wider than long, and with the outward anterior corner rounded and an anterior strip with much less sculpture (usually just under half as long as the sculptured area posterior to it). T3 completely smooth. Ovipositor sheaths short, at most one-quarter the length of metathorax, with a long seta on the apex. Fore wing with triangular areolet, its posterior side in straight line with vein r.

All European specimens examined have T1 mostly smooth and shiny, except for the apical 0.2–0.3, which is moderately rugose and with a weak striate area directed inwards posteriorly; and T2 tends to be much shorter, sometimes with only the elevated median field heavily sculptured, while the rest of the tergite is weakly sculptured or sometimes almost smooth. All the Canadian specimens have a uniformly sculptured, longitudinally striated T1; and T2 mostly rugose (both median and lateral fields). The mesoscutum and scutellum tend to be smoother in European specimens compared with the Canadian ones, although the differences are subtle.

**Male.** As for female, but T1 narrows apically a bit more, and the posterior part of the sides of T2 are substantially shinier and less sculptured than the elevated median field.

**Comments.** Most of the specimens from Europe (except for some from moderately northern

**Fig. 2.** Haplotype network of COI DNA barcodes of *Diolcogaster claritibia* using a probability of parsimony of 0.93. Seven of the sampled haplotypes (HT) were found in only one specimen and are each represented by an oval. One haplotype, represented by a small rectangle, was found in a specimen from Cyprus and a specimen from France. One haplotype, represented by a large square, was found in 69 Canadian specimens. Small circles represent unsampled intermediate haplotypes and lines indicate single sequence differences (mutations) joining haplotypes.



latitudes) and the Middle East are more lightly sculptured than those from Canada. A possible explanation for this is that the former come from hotter regions.

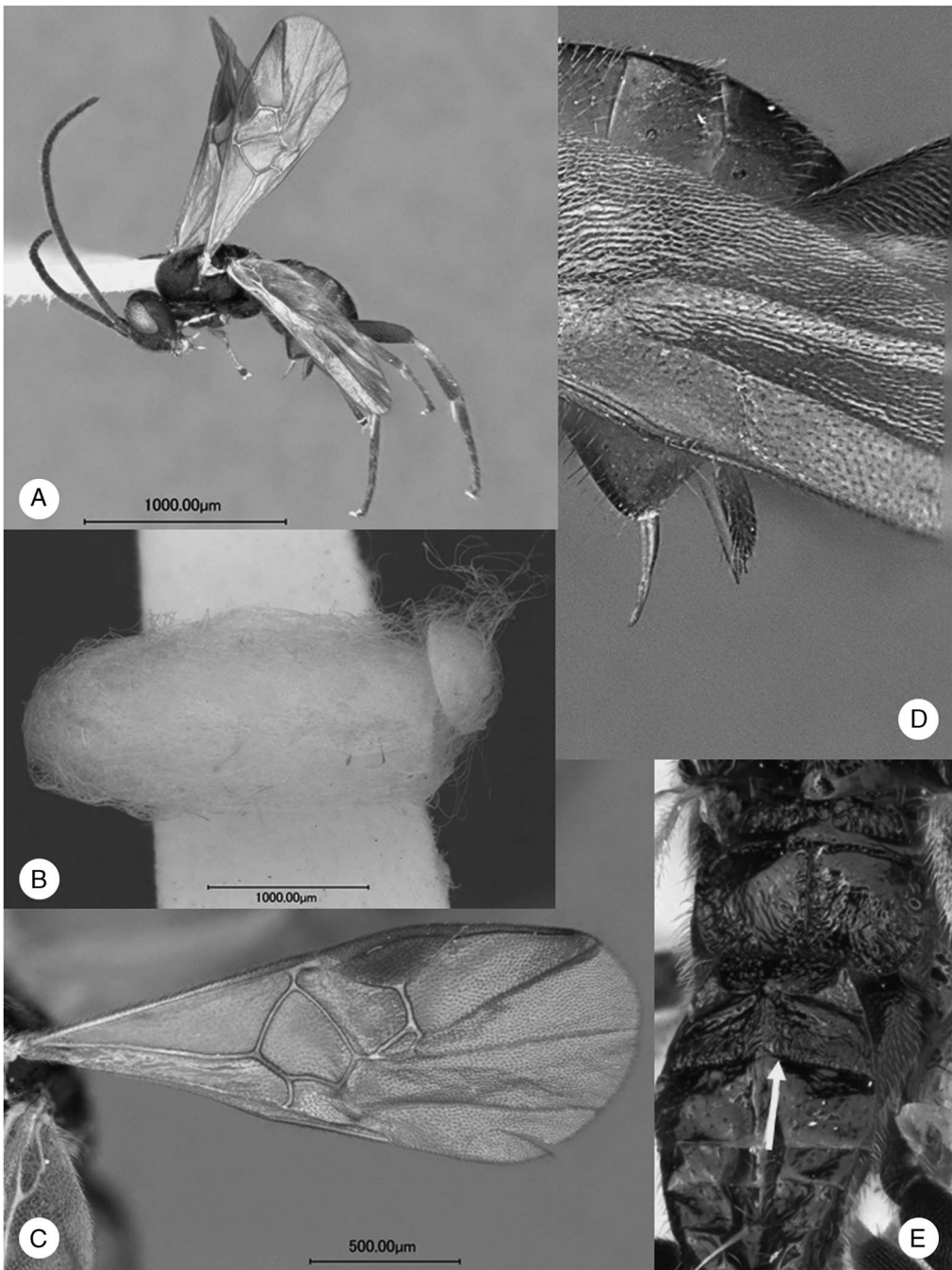
Nixon (1965, fig. 311) illustrated the basal tergites of the holotype of his *Protomicroplitis orontes* Nixon, 1965, a synonym of *D. claritibia* (see below). In the European specimens we examined, including the holotypes of both *P. orontes* and *Microgaster claritibia*, the sculptured side areas of mediotergite 2 are not as hugely transverse as depicted in Nixon’s paper, but viewed from in front there is a wide smooth area in front of the sculptured sides, both being of somewhat variable shape.

**Molecular analysis.** In the neighbour-joining tree, the Canadian *Diolcogaster* specimens cluster with the European *D. claritibia* specimens. The European specimens do not form a cluster distinct from the Canadian specimens. The average pairwise tree distance among the Canadian

*Diolcogaster* and the European *D. claritibia* members was 1.8% when only one representative per haplotype found in the haplotype network analysis was included (Fig. 1) and 0.3% when all sequences were included. The average pairwise distance between *D. claritibia* specimens and members of the next closest species was 11.4% (12% when all sequences were included). The small ratio of intraspecific to interspecific distance indicates that genetic differences within the putative *D. claritibia* are small relative to differences between them and members of the closest species included in the analysis. In the Bayesian MCCT, the Canadian and European *D. claritibia* specimens formed a monophyletic group supported by a posterior probability of 1 (Fig. 5). Again, the European and Canadian specimens did not form distinct monophyletic groups.

The haplotype network analysis, using a probability of parsimony of 0.95, recovered

**Fig. 3.** *Diolcogaster claritibia*, specimens from Canada. (A) Habitus. (B) Cocoon. (C) Fore wing. (D) Hypopygium, ovipositor and ovipositor sheaths. (E) Mediotergite 2 (indicated by yellow arrow) and propodeum in the background.

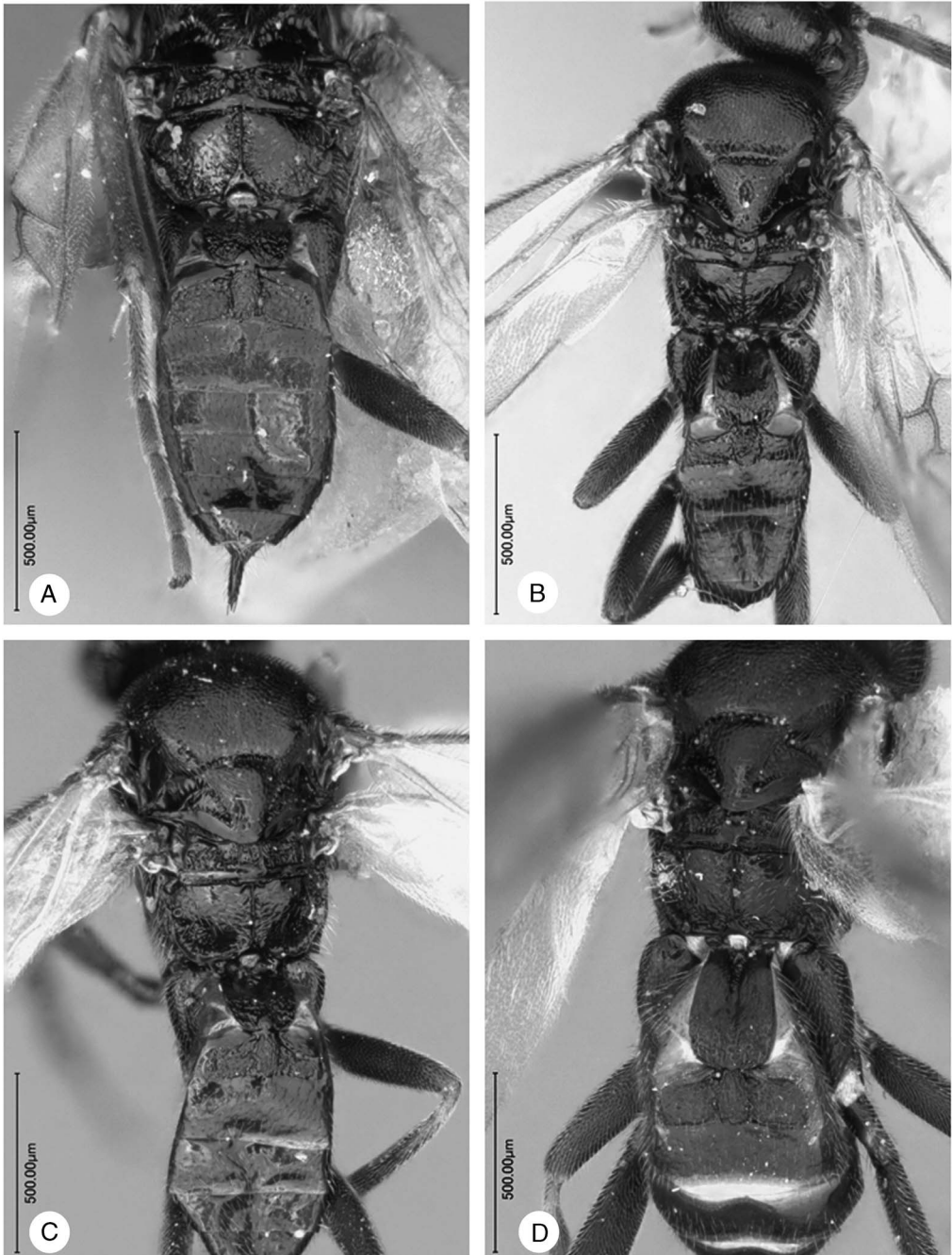


two distinct mitochondrial lineages, one including specimens from Cyprus and France, and the other specimens from Turkey, the Netherlands,

and Canada. Therefore, a second analysis was run using a probability of parsimony of 0.93 to join all haplotypes into one network (Fig. 2).



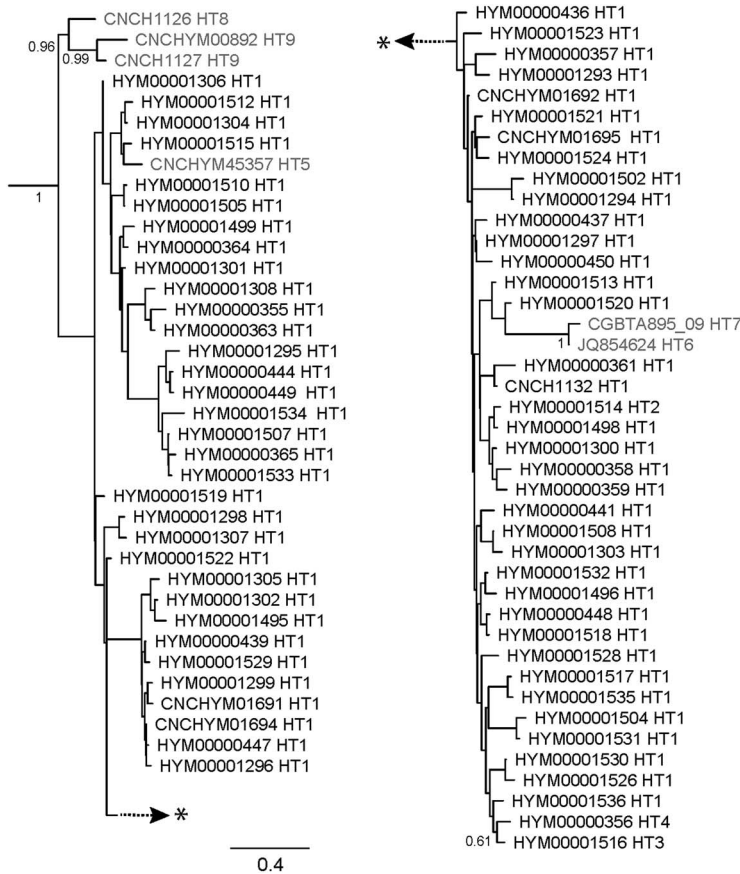
**Fig. 4.** *Diolcogaster claritibia* dorsal view of mesosoma and metasoma showing propodeum and meditergites. (A) Specimen from Cyprus. (B) Specimen from France. (C) Specimen from Jordan. (D) Specimen from Alberta, Canada.



This suggests that the Canadian specimens should not be considered a distinct species from the European specimens, but that there may be

distinct mitochondrial lineages within Europe. The latter could be an artefact of very limited sampling within Europe.

**Fig. 5.** Bayesian Maximum Clade Credibility Tree of COI DNA barcodes of *Diolcogaster claritibia*. Voucher codes followed by haplotype # are shown in grey for Palaearctic specimens and in black for Canadian specimens. Outgroups are not shown and posterior probabilities above 0.5 are shown below branches. Asterisks and arrows indicate continuation of the tree.



The available data indicate that the European and Canadian specimens should be considered one species for now, but more specimens and loci need to be sampled to conclusively determine the phylogeographic history of the species. The small number of base pair differences seen within the Canadian specimens compared to within the European specimens, the phylogeny, and the haplotype networks, suggest that *D. claritibia* was fairly recently introduced to North America from Europe.

**The identity of *Protomicropititis orontes* Nixon, 1965**

In view of the small morphological and molecular differences found between Canadian and

European/Middle East specimens, we included in our study considerations of a further nominal species from Europe: *D. orontes* (Nixon 1965), originally described from Finland in the genus *Protomicropititis* but later treated as a synonym of *D. claritibia* by Papp (1981). Because it was not clear that Papp's (1981) synonymy was backed by an examination of the holotype of *P. orontes*, we examined the holotypes of both nominal taxa. In the holotype of *P. orontes* the sculptured part of the T2 is divided into three areas – a central longitudinal area flanked by lateral areas. The shape and length of the lateral sculptured areas (situated posteriorly to a polished area) differs appreciably between the two holotype specimens. However, both fall within the range of variation

seen in the extensive material of *D. claritibia* from Canada, which includes dozens of specimens with similar barcodes. For example, some specimens from Lethbridge, Alberta, Canada, have this sculptured area considerably shorter laterally than medially (corresponding to the condition in the holotype of *P. orontes* (see Nixon 1965, fig. 311), thus differing from some specimens from Ottawa, Ontario, Canada, in which this sculptured area is virtually as long laterally as medially – corresponding to the condition in the holotype of *Microgaster claritibia* (see Papp 1959, fig. 15). Thus, although the condition of T2 in the holotype of *P. orontes* is at the edge of the range of variation seen in the European/Middle Eastern specimens that we have examined, we concur with the synonymy proposed by Papp (1981): *M. claritibia* (Papp, 1959), senior synonym = *P. orontes* Nixon, 1965, junior synonym.

Based on the available morphological and molecular evidence, we consider all specimens examined as belonging to the same species, *D. claritibia*. With the new records included here, the species is considered likely to be widely distributed in the Nearctic region as well as the Palaearctic.

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## References

- Clement, M., Posada, D., and Crandall, K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**: 1657–1660.
- Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**: 772.
- Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., *et al.* 2011. Geneious v6.0.5. Available from [www.geneious.com](http://www.geneious.com) [accessed 30 September 2013].
- Huber, J.T. and Sharkey, M.J. 1993. Structure. In *Hymenoptera of the world: an identification guide to families*. Monograph No. 1894E. Edited by H. Goulet and J.T. Huber. Agriculture Canada Research Branch, Ottawa, Ontario, Canada. Pp. 13–59.
- Hudson, R.R. 1990. Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology*, **7**: 1–44.
- Ivanova, N.V., Dewaard, J.R., and Hebert, P.D.N. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, **6**: 998–1002. doi:10.1111/j.1471-8286.2006.01428.x.
- Mason, W.R.M. 1981. The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. *Memoirs of the Entomological Society of Canada*, **115**: 1–147.
- Nixon, G.E.J. 1965. A reclassification of the tribe Microgasterinae (Hymenoptera: Braconidae). *Bulletin of the British Museum (Natural History)*, Entomology supplement, **2**: 1–284.
- Papp, J. 1959. The *Microgaster* Latr., *Microplitis* Först., and *Hygroplitis* Thoms., species of the Carpathian basin (Hymenoptera, Braconidae). *Annales Historico-naturales Musei Nationalis Hungarici*, **51**: 397–413.
- Papp, J. 1981. Contributions to the braconid fauna of Hungary, III. Opiinae and Microgasterinae (Hymenoptera: Braconidae). *Folia Entomologica Hungarica*, **42**: 127–141.
- Rambaut, A. and Drummond, A.J. 2009. Tracer v1.5 [online]. Available from <http://beast.bio.ed.ac.uk/Tracer> [accessed 30 September 2013].
- Rambaut, A. and Drummond, A.J. 2013. TreeAnnotator v1.7.0 [online]. Available from <http://beast.bio.ed.ac.uk> [accessed 30 September 2013].
- Ronquist, F. and Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.
- Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**: 406–425.
- Shaw, M.R. 1994. Parasitoid host ranges. In *Parasitoid community ecology*. Edited by B. A. Hawkins and W. Sheehan. Oxford University Press, Oxford, United Kingdom. Pp. 111–144.
- Smith, M.A., Fernández-Triana, J.L., Eveleigh, E., Gómez, J., Guclu, C., Hallwachs, W., *et al.* 2013. DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly 20 000 sequences. *Molecular Ecology Resources*, **13**: 168–176. doi:10.1111/1755-0998.12038.
- Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A. R., Janzen, D.H., Hallwachs, W., *et al.* 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences*, **105**: 12359–12364. doi:10.1073/pnas.0805319105.

- Smith, M.A., Wood, D.M., Janzen, D.H., Hallwachs, W., and Hebert, P.D.N. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences*, **104**: 4967–4972. doi:10.1073/pnas.0700050104.
- Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W., and Hebert, P.D.N. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences*, **103**: 3657–3662. doi:10.1073/pnas.0511318103.
- Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**: 512–526.
- Templeton, A.R., Crandall, K.A., and Sing, C.F. 1992. A cladistics analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**: 619–633.
- Whitfield, J.B. 1997. Subfamily Microgastrinae. *In* *Manual of the New World genera of the family Braconidae (Hymenoptera)*. Special Publication No. 1. *Edited by* R.A. Wharton, P.M. Marsh, and M.J. Sharkey. International Society of Hymenopterists, Washington, DC, United States of America. Pp. 333–364.
- Yu, D.S.K., van Achterberg, C., and Horstmann, K. 2012. Taxapad 2012, Ichneumonoidea 2011. Database on flash-drive. Ottawa, Ontario, Canada. Available from [www.taxapad.com](http://www.taxapad.com) [accessed 9 February 2014].