

Contributions to the study of the Holarctic fauna of Microgastrinae (Hymenoptera, Braconidae). I. Introduction and first results of transatlantic comparisons

Jose Fernandez-Triana¹, Mark R. Shaw², Sophie Cardinal¹, Peter G. Mason¹

1 Canadian National Collection of Insects, Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, ON K1A 0C6, Canada **2** Honorary Research Associate, National Museums of Scotland, Chambers Street, Edinburgh EH1 1JF, U.K.

Corresponding author: Jose Fernandez-Triana (jfr triana@uoguelph.ca)

Academic editor: G. Broad | Received 3 February 2014 | Accepted 4 March 2014 | Published 28 March 2014

Citation: Fernández-Triana J, Shaw MR, Cardinal S, Mason PG (2014) Contributions to the study of the Holarctic fauna of Microgastrinae (Hymenoptera, Braconidae). I. Introduction and first results of transatlantic comparisons. Journal of Hymenoptera Research 37: 61–76. doi: 10.3897/JHR.37.7186

Abstract

Specimens of Microgastrinae (Hymenoptera: Braconidae) from both sides of the Holarctic region (Nearctic and Palaearctic) were sampled for DNA barcoding and examined morphologically. Two species are recorded for the first time for the Nearctic: *Apanteles brunnistigma* Abdinbekova, and *Microgaster raschkiellae* Shaw. Another European species, *Apanteles xanthostigma* (Haliday), previously introduced as a biological control agent, is confirmed to be present in North America. For another 13 species significant range extension is documented, including new records for France, Canada, United States, and Sweden. New host data are also provided for several species. The species name *Apanteles masmithi* Fernández-Triana is considered a **syn. n.** of *Dolichogenidea britannica* (Wilkinson).

Keywords

Microgastrinae, DNA barcoding, morphology, Holarctic, Palaearctic, Nearctic

Introduction

Microgastrinae wasps (Hymenoptera: Braconidae) are one of the most important groups in the biological control of Lepidoptera caterpillar pests of forestry and agriculture (Whitfield 1997). They are also the second largest subfamily of Braconidae, with 2,233 described species (Yu et al. 2012), and many thousands more awaiting description (e.g. Mason 1981; Rodriguez et al. 2012).

According to the latest version of Taxapad (Yu et al. 2012) there are 757 species of Microgastrinae in the Palaearctic (529 of them recorded from the Western Palaearctic, and 496 from the Eastern Palaearctic), and 320 described species in the Nearctic (for the limits of the Nearctic and Palaearctic regions, we follow O'Hara et al. 2009).

Altogether, the Nearctic plus the Palaearctic (i.e., the Holarctic region) have 1029 species, accounting for 46% of the described microgastrines worldwide. This, of course, is an artifact due to most studies being historically focused on the north-temperate areas of the planet. There is a significant diversity waiting to be described in the tropics, totalling several thousand new species (e.g., Rodriguez et al. 2012). Regardless of that, the information currently available for the Holarctic allows for some analyses and comparisons to be made.

While the Palaearctic has twice the number of known species as the Nearctic, the final figures might be much closer. Most of the European (i.e. Western Palaearctic) species are already known, with the remaining diversity to be discovered being mostly morphologically cryptic species. On the other hand, the Eastern Palaearctic is much less studied, and it is likely that a significant number of additional species remain to be found and described there, although we are not aware of any published information providing estimates. The most updated list of species for the Palaearctic can be found in *Fauna Europaea* (van Achterberg 2012), although that list uses a more condensed generic classification than the one followed here, which is based on Mason (1981) largely as interpreted by Papp (1988).

As for the Nearctic region, it is clear that a considerable number of species are still unknown. For example, Fernández-Triana (2010) estimated that just for the northern part of the Nearctic (i.e. Canada and Alaska) the known species (approximately 200) only represent half of the actual diversity. For the southern Nearctic, which is warmer and more diverse, it is reasonable to expect that 50% or more of the species remain unknown and/or undescribed there too. Clearly there is still much to be discovered, even in the relatively poorly diverse, temperate regions. The most updated lists of species for the Nearctic can be found in Whitfield (1995) and Fernández-Triana (2010).

According to the published data, only 272 (26%) of the 1,029 Holarctic species of Microgastrinae are shared between the Nearctic and Palaearctic regions. This relatively low percentage might be due to some taxa being described twice, as different species, on the two sides of the Atlantic. The large number of descriptions, and the holotypes being scattered across a large number of collections, make it very difficult to approach the study of this group from a truly Holarctic perspective. Thus, taxonomic revisions

of Microgastrinae have usually focused on either the Palaearctic or the Nearctic, with very few studies covering both regions.

DNA barcoding uses a short standardized region of the mitochondrial gene cytochrome *c* oxidase (COI) as a key character for species-level identification and discovery (Floyd et al. 2002; Hebert et al. 2003a; Hebert et al. 2003b; Janzen et al. 2009; Smith et al. 2006; Smith et al. 2007; Smith et al. 2008). DNA barcoding has been extensively used in biodiversity and taxonomic studies of Microgastrinae during the past few years (summarized in Fernández-Triana et al. 2014), due to the recent availability of over 20,000 sequences from more than 75 countries (e.g., Smith et al. 2013). Those resources have allowed us to compare sequences of specimens from different areas and unravel potentially new distribution patterns – which were previously overlooked, or unknown until now. All new distribution records reported here have been corroborated through careful comparisons with holotypes or authenticated specimens deposited in major collections.

Methods

This paper is mostly based in the study of DNA barcoded specimens of Microgastrinae from the Canadian National Collection, Ottawa (CNC), and the National Museums of Scotland, Edinburgh (NMS). Additionally, some barcoded specimens from the Swedish Malaise Trap Project (SMTP) were available for study; the SMTP aims to provide species determinations for all the 80 million insect specimens obtained from Malaise traps sampling at a wide range of landscapes and habitats in Sweden (<http://www.stationlinne.se/en/research/the-swedish-malaise-trap-project-smtp/>). Pictures of barcoded specimens housed in the Biodiversity Institute of Ontario, Guelph were also analyzed. Examination of holotypes deposited in the National Museum of Natural History, Washington D.C. (NMNH), and the Natural History Museum, London (BMNH), was made in some cases – mostly when necessary to verify identifications of specimens in the CNC and NMS.

One of us (MRS) provided samples of reliably determined Microgastrinae from Europe for barcoding, reared from identified hosts that are in many cases the very hosts from which the species in question was originally described. This provides a framework of fixed reference points from which to assess the specific identity of other specimens, whether reared or not and from both the Nearctic and the Palaearctic, initially through their barcodes. When host names are given for specimens in NMS, we append “Det.” and then the name of the person who reared the caterpillars, because it was (s)he who was responsible for the host determination.

To uncover new distribution patterns, we first scanned the tree presented in Smith et al. (2013), which contained all Microgastrinae sequences available at the time of its publication, and searched for clusters of closely related sequences (2% divergence or less) that contained both identified and unidentified specimens. These unidentified specimens were then morphologically studied to see if they were the same species as the identified specimens. To further uncover new distribution patterns, we generated

new barcode sequences from authenticated material from the NMS and CNC with the hope that some of these sequences would cluster with unidentified sequences found in BOLD. We used the Identification Engine tool (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) in the Barcode of Life Data Systems (BOLD) to compare our newly generated sequences to all sequences available in BOLD. When our sequences matched previously unidentified sequences with a probability of placement above 98%, we morphologically studied the specimens to see if they were the same species. In some cases, unidentified specimens differed morphologically from the identified specimens they were clustering with based on molecular barcoding data. These cases will be studied further and results will be published in following papers.

Newly generated DNA barcodes were obtained using DNA extracts prepared from single legs using a glass fibre protocol (Ivanova et al. 2006). Extracts were re-suspended in 30 µl of dH₂O, and a 658-bp region near the 5' terminus of the COI gene was amplified using standard primers (LepF1–LepR1) following established protocols (Smith et al. 2006; Smith et al. 2007; Smith et al. 2008). All newly generated sequences were edited in Geneious v.6.1.6 created by Biomatters (<http://www.geneious.com/>) and submitted to Genbank (see Table 1 for Genbank accession numbers). All sequences from Smith et al. (2013) can also be retrieved from BOLD (Ratnasingham and Hebert 2007).

Sequences were considered as “barcode-compliant” when they had 500 or more base pairs and had less than 1% ambiguous characters (Barcode Compliance standards as in http://www.boldsystems.org/index.php/resources/handbook?chapter=6_managingdata.html§ion=record_list).

Genera (and species within a genus) are presented in alphabetical order. Each new distribution record is discussed within the context of the previously known distribution of the species. For Canadian provinces and territories, and states of the United States we use acronyms consisting of two capital letters following Canada Post (<http://www.canadapost.ca/tools/pg/manual/PGaddress-e.asp>).

Table 1. Details of specimens from the National Museums of Scotland (NMS) with DNA barcode sequences newly obtained – and not yet available in the Barcode of Life Data Systems (BOLD). Collection codes for NMS and the Canadian National Collection of Insects (CNC), as well as GenBank accession numbers are included.

Wasp species	Host species	Locality	Emergence date	NMS code	CNC code	GenBank accession numbers
<i>Apanteles brunnistigma</i>	<i>Pyausta aurata</i>	France: Aveyron, Livinhac-le-Haut	18.vi.2012	MRS 0183	CNCHYM49298	KJ459123
<i>Apanteles brunnistigma</i>	<i>Pyausta aurata</i>	France: Aveyron, Livinhac-le-Haut	11.vi.2012	MRS 0184	CNCHYM49319	KJ459223
<i>Dolichogenidea britannica</i>	<i>Ptocheuusa paupella</i>	England: Hants, Portsmouth	vii.2011	MRS 0217	CNCHYM45321	KJ459126
<i>Microgaster raschkiellae</i>	<i>Mompha raschkiella</i>	Scotland: Skye, Armadale	4.vii.2012	MRS 0192	CNCHYM45380	KJ459161
<i>Pbrolesor viminetorum</i>	<i>Elachista poae</i>	England: W. Yorks, Copley	28.iv.2012	MRS 0166	CNCHYM45332	KJ459179

Photos were taken with a Keyence VHX-1000 Digital Microscope, using a lens with a magnification range of 13–130×. Multiple images through the focal plane were taken of a structure and these were combined to produce a single in-focus image, using the software associated with the Keyence System.

Results

Two species previously known only from the Palaearctic are here recorded for the first time for the Nearctic. Another European species, previously introduced as a biological control agent but not known to be established, is here confirmed to be still present in North America. Additionally, another 13 species have been found to have a wider range than previously known – in some cases the new data reported here significantly expand the known distributions.

Most of the range expansions recorded were towards northern areas (e.g. Alaska, Manitoba, Nunavut, and Yukon Territory) and may reflect an increase in the availability of material recently collected in arctic and sub-arctic localities of North America (e.g., Fernández-Triana et al. 2011; Stahlhut et al. 2013). Other new records fill gaps in the known distribution of species.

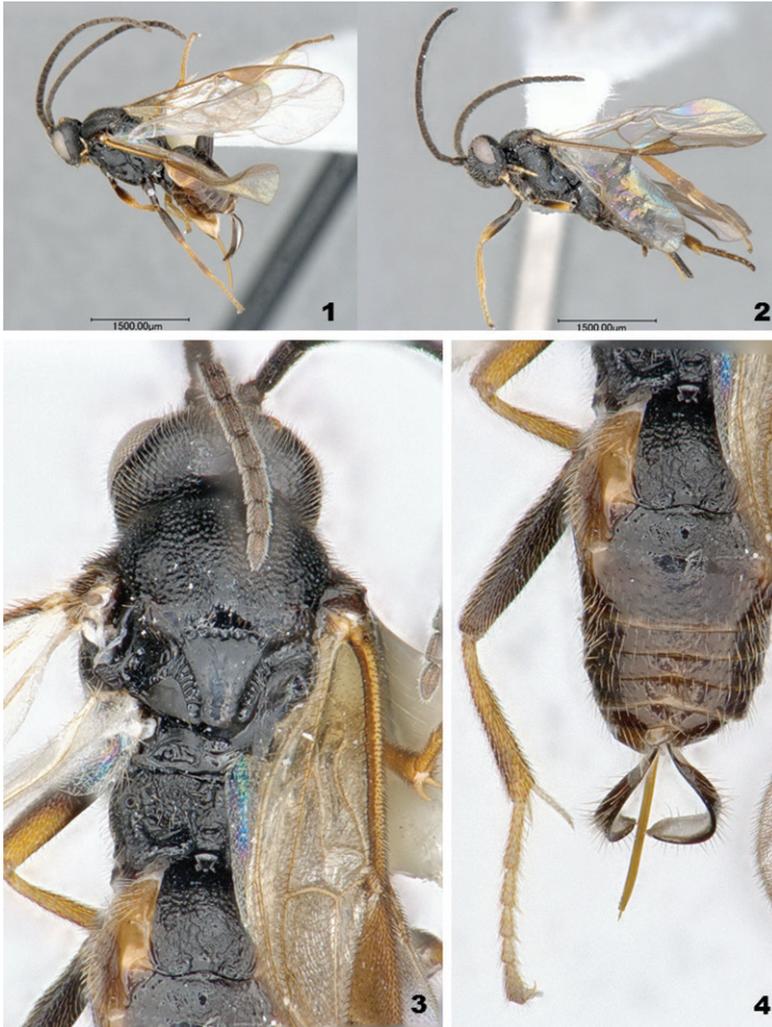
1) *Apanteles brunnistigma* Abdinbekova, 1969

Figs 1–4

Previously, this species was known to be widely distributed in the Palaearctic (Yu et al. 2012). Here it is recorded for the first time for the Nearctic (**Canada**: MB, Churchill, 51 specimens; and NL, Cap St-George, one specimen). The specimens from Manitoba were named as “*Apanteles jft02*” in a previous paper (Fernández-Triana et al. 2011). In the Palaearctic it is recorded for the first time from **Sweden** (all from the SMTP: Haninge Municipality, Stockholm County, one specimen; and Gällivare Municipality, Norrbotten County, four specimens) and from **France** (Aveyron, Livinhac-le-Haut, two specimens from the NMS).

Apanteles brunnistigma had been previously recorded as a parasitoid of Depressariidae (*Agonopterix umbellana* (Fabricius), *Depressaria ultimella* Stainton), and Tortricidae (*Aphelia viburnana* (Denis & Schiffermüller), *Archips rosana* (Linnaeus), *Eucosma rubescana* (Constant) (as *catoptrana*), and *Gynnidomorpha vectisana* (Humphries and Westwood)), as summarized in Yu et al. (2012). Specimens in the NMS were reared from the following (all of them are new host records): Epermeniidae (*Epermenia chaerophyllella* (Goeze) (Det. A. N. B. Simpson)), Tortricidae (*Rhopobota naevana* (Hübner) (Det. J. M. Chalmers-Hunt)), and Crambidae (*Pyrausta aurata* (Scopoli) (Det. R. J. Heckford, M. R. Shaw)).

We analyzed 61 barcode sequences available for this species (59 of them barcode compliant), representing 11 haplotypes from Canada, France, Sweden, and Ukraine.



Figures 1–4. *Apanteles brunnistigma*. **1** Habitus, lateral view, Swedish specimen with CNC code CNCH1798 **2** Habitus, lateral view, Canadian specimen with CNC code 07PROBE-23429 **3** Head, mesosoma and mediotergites 1-2, dorsal view, specimen CNCH1798 **4** Metasoma, dorsal view, specimen CNCH1798.

The difference between haplotypes ranged from one to 10 base pairs (0.2–1.5%), with most sequences differing by five base pairs or less.

2) *Apanteles ensiger* (Say, 1836)

This species was known to be widely distributed in the Nearctic (Yu et al. 2012). Here it is recorded for the first time, from numerous specimens, in five additional

provinces/territories of Canada (AB, Banff National Park; NB, Kouchibouguac National Park; NL, Corner Brook; Plum Point; St-Andrew's; St-David's; NT, Wood Buffalo National Park; PE, Blooming Point; and SK, Prince Albert National Park), and three states of the United States (AK, Anchorage; Trapper Creek, Petersville Road at 62°N; FL, Kissimmee Prairie Preserve State Park; and MA, Barnstable Country, Woods Hole). The records from Alaska considerably expand westwards and northwards the known distribution of the species.

This species had been previously recorded from the Lepidoptera families Tortricidae (*Choristoneura occidentalis* Freeman), and Crambidae (*Fissicrambus mutabilis* (Clemens), and *Neodactria zeella* (Fernald)), as summarized in Yu et al. (2012). Here a new host from Tortricidae is recorded, *Epiblema strenuana* (Walker) from one Canadian specimen (ON, Grimsby) deposited in the CNC.

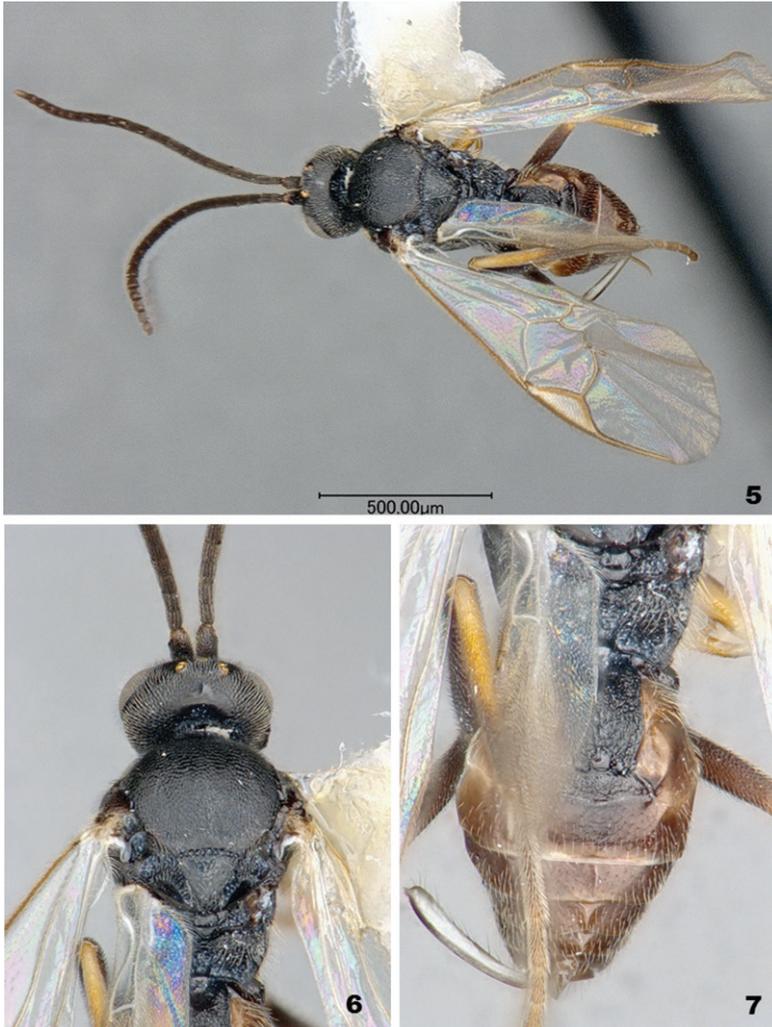
We analyzed 178 barcode sequences available for this species (166 of them barcode compliant), representing 20 haplotypes from Canada and the United States. The difference between haplotypes ranged from one to eight base pairs (0.2–1.2%).

3) *Apanteles xanthostigma* (Haliday, 1834)

Figs 5–7

Previously, the species was known to be widely distributed in the Palaearctic (Yu et al. 2012), with one record from tropical Africa (De Saeger 1944). In 1963, 290 specimens were released in St. John's, Newfoundland, Canada (Williamson 1963); however, until now it was not known if the species became established there (Fernández-Triana 2010). Here we confirm for the first time its continued presence in the Nearctic, from 17 specimens collected in three provinces of western Canada (MB, Churchill; SK, Prince Albert National Park; and BC, Sorrento). The specimens from Manitoba were named as "*Apanteles jft03*" in a previous paper (Fernández-Triana et al. 2011).

In the NMS there are authenticated specimens reared from the following Lepidoptera families and species: Choreutidae (*Choreutis diana* (Hübner) (R. J. Heckford)), Gracillariidae (*Caloptilia betulicola* (Hering) (Det. S. D. Beavan, K. P. Bland, R. J. Heckford, J. R. Langmaid, M. R. Shaw, P. A. Sokoloff), *Caloptilia elongella* (Linnaeus) (Det. K. P. Bland, M. R. Shaw), *Caloptilia stigmatella* (Fabricius) (Det. P. J. Johnson, J. R. Langmaid, S. E. Whitebread), *Povolnya leucapennella* (Stephens) (Det. R. J. Heckford) new host record, *Parornix devoniella* (Stainton) (Det. M. R. Shaw) new host record, and *Parornix scoticella* (Stainton) (Det. M. R. Shaw) new host record), Pyralidae (*Acrobasis suavella* (Zincken) (Det. M. F. V. Corley, R. J. Heckford) new host record), Tortricidae (*Acleris hastiana* (Linnaeus) (Det. P. J. Johnson) new host record, *Adoxophyes orana* (Fischer von Röslerstamm) (Det. A. Wilson), *Rhopobota naevana* (Hübner) (Det. M. R. Young)), and Yponomeutidae (*Swammerdamia caesiella* (Hübner) (Det. M. R. Shaw), *Swammerdamia pyrella* (Villers) (Det. J. L. Gregory), *Paraswammerdamia albicapitella* (Scharfenberg) (Det. J. L. Gregory, N. Hall)).



Figures 5–7. *Apanteles xanthostigma*. **5** Habitus, dorsal view, Swedish specimen with CNC code CNCH1804 **6** Head and mesosoma, dorsal view, specimen CNCH1804 **7** Propodeum and metasoma, dorsal view, specimen CNCH1804.

Yu et al. (2012) summarized recorded hosts for *A. xanthostigma*, including 11 families and 63 species of Lepidoptera. Even allowing for the probability that some records are erroneous (as must surely be the case for the two records from Diptera (Cecidomyiidae)), the historical data demonstrate an extremely broad host range comprising semi-concealed arboreal microlepidoptera larvae feeding on the foliage of trees and shrubs. Including the new records reported here, there are now 68 host species of Lepidoptera recorded, with the best represented host families being Tortricidae (28 species), Gracillariidae (14), Yponomeutidae (6), Gelechiidae (4), Pyralidae (4), and Choreutidae (3) – a pattern of representation that is to a rather large extent just a

reflection of the number of host species available to the parasitoid within its essentially ecologically defined host range.

We analyzed 12 barcode sequences available for this species (10 of them barcode compliant), representing four haplotypes from Canada, the Netherlands, Russia, and Sweden. The difference between haplotypes ranged from one to eight base pairs (0.2–1.2%), with most sequences differing by six base pairs or less.

The fact that new Canadian records come from several localities, all far apart (more than 3,000 km) from the original site of introduction in Newfoundland, suggests that the species was probably already established in North America prior to the 1963 introduction.

4) *Cotesia crambi* (Weed, 1887)

This species was previously known from northeast and central United States (Yu et al. 2012) and one locality in Canada (QC, Frelighsburg) (Fernández-Triana et al. 2009). Here it is recorded for a second locality and province in Canada (ON, Ottawa, three specimens). Three species of Crambidae have been recorded as hosts of the species (Yu et al. 2012).

We analyzed six barcode sequences available for this species (two of them barcode compliant), representing 3 haplotypes from Canada and the United States. The difference between haplotypes was one base pair (0.2%).

5) *Cotesia parastichtidis* (Muesebeck, 1921)

Previous records show a wide distribution within the Nearctic, although records are scarce and sparse over North America. Here it is recorded for the first time in three additional Canadian provinces/territories (AB, Waterton Lakes National Park, one specimen; MB, Churchill, four specimens; and YT, Top of the World Highway, km 82, one specimen) and one state of the United States (AK, Anchorage, three specimens). The new records expand northward the known distribution of the species. Some of the specimens from Manitoba were named as “*Cotesia jft04*” in a previous paper (Fernández-Triana et al. 2011). Five species within three families of Lepidoptera (Geometridae, Noctuidae, and Tortricidae) have been recorded as hosts of the species (Yu et al. 2012).

We analyzed 16 barcode sequences available for this species (seven of them barcode compliant), representing five haplotypes from Canada. The difference between haplotypes ranged from one to 13 base pairs (0.2–2.0%).

6) *Cotesia rufocoxalis* (Riley, 1881)

This species was previously known from eastern and central United States and the Canadian province of Nova Scotia. Here it is recorded for the first time for two additional states of the United States (NJ, Metuchen, one specimen; and AL, Mobile County,

Country Road 1, one specimen). Five species of Lepidoptera, one in Lasiocampidae and four in Noctuidae, have been recorded as hosts of the species (Yu et al. 2012).

We analyzed six barcode sequences available for this species (two of them barcode compliant), representing 3 haplotypes from Canada and the United States. The difference between haplotypes ranged from one to three base pairs (0.2–0.5%).

7) *Cotesia selenevora* Shaw, 2009

This species was previously known from Belgium and Finland. Here recorded for the first time for Sweden (Småland, Nybro kommun, Bäckebo, Grytsjöns naturreservat, Lat/Lon: 63.1766, 15.3005, one specimen from the SMTP).

The reared material (the type series) in NMS is from *Boloria selene* (Dennis & Schiffermüller) (Lepidoptera: Nymphalidae) (C. Turlure, J. Chouett), so far the only known host of *C. selenevora*. There are no records additional to those given by Shaw (2009).

We analyzed two barcode sequences available for this species (both barcode compliant), representing 1 haplotype from Belgium and Sweden.

8) *Dolichogenidea britannica* (Wilkinson, 1941)

Apanteles masmithi Fernández-Triana, 2010. **Syn. n.**

After comparing the type material of *Dolichogenidea britannica* and *Apanteles masmithi*, we conclude that both are the same species – and thus the latter name becomes a synonym of the first. This represents the first record of *D. britannica* for the Nearctic. The evidence from morphology is also supported by DNA barcoding – which indeed had suggested the synonymy in the first place. The difference was only three base pairs (0.5%) between the two available haplotypes (one haplotype from eight Canadian specimens (three specimens barcode compliant), the other haplotype from one English specimen (barcode compliant) reared from the same host species as the type). This species is an example of the need to study the Holarctic fauna as a whole, whenever possible; otherwise the same species is likely to be described twice (or more) on both sides of the Atlantic.

The type series of *D. britannica* was reared from the gelechiid now known as *Ptocheuusa paupella* (Zeller). An additional host record given by Yu et al. (2012) is from Tortricidae (*Enarmonia formosana* (Scopoli)). Reared material in NMS is from Gelechiidae (*Ptocheuusa paupella* (Det. E. S. Bradford, J. R. Langmaid, M. S. Parsons, P. A. Sokoloff), almost certainly *Isophrictis striatella* (Dennis and Schiffermüller) (Det. E. C. Pelham-Clinton) new host record), and Cosmopterigidae (*Limnaecia phragmitella* Stainton (Det. B. Goodey, I. Sims, P. A. Sokoloff, I. A. Watkinson) new host record of family and species). It is noteworthy that some of the Canadian specimens were also reared from *L. phragmitella*.

9) *Dolichogenidea melanopa* (Viereck, 1917)

This species was previously known in the Nearctic from three rather separate areas (CT in the United States, BC and PE in Canada). Here it is recorded for the first time, from numerous specimens, from six additional provinces/territories in Canada (AB, Banff National Park; MB, 22 km S of Camperville; Churchill; 1 km N of Winnipeg; NL, Corner Brook; Port Saunders; St-Andrew's; QC, Belle-Anse; SK, Grasslands National Park; and YT, Whitehorse); and one state in the United States (AK, S of Anchorage). The new records show the species is rather widely distributed within the Nearctic north of 40°N. Specimens were previously named as *Dolichogenidea* jft09 in Fernández-Triana et al. (2010). The only recorded host is *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae).

We analyzed 70 barcode sequences available for this species (64 of them barcode compliant), representing 12 haplotypes from Canada and the United States. The difference between haplotypes ranged between one and three base pairs (0.2–0.5%).

10) *Microgaster raschkiellae* Shaw, 2012

Figs 8–12

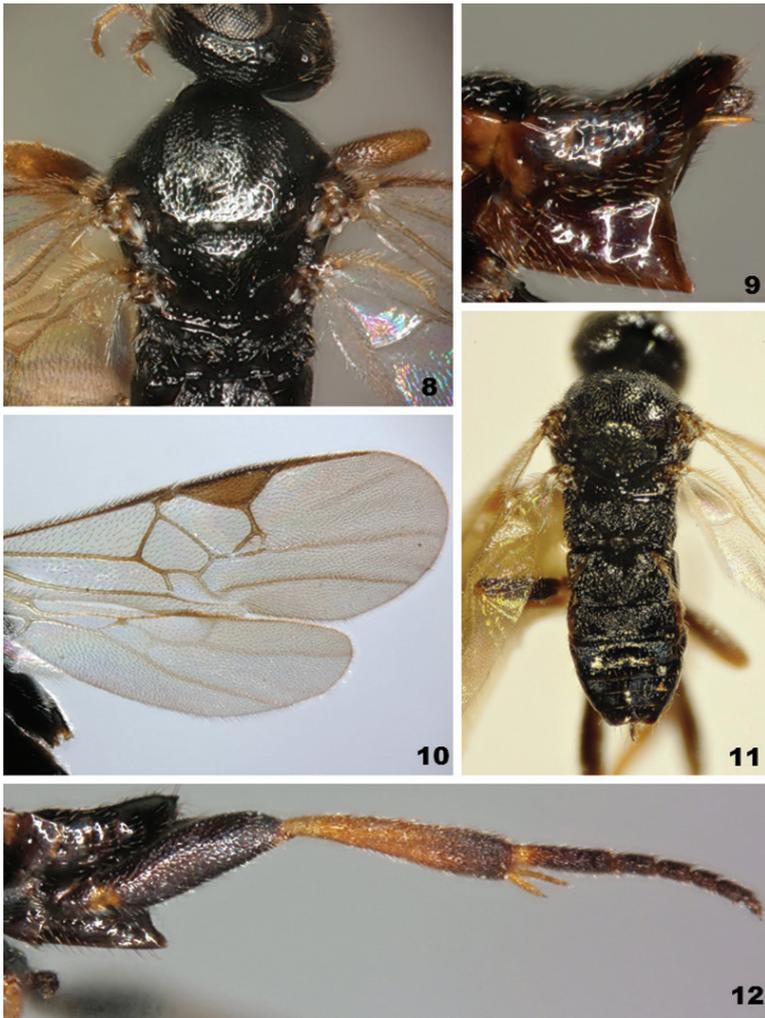
This species was recently described from Great Britain (England, Scotland and Wales), where it was widely recovered as the commonest parasitoid of *Mompha raschkiella* (Zeller) (Lepidoptera: Momphidae). Because it has not been reared from any other species of *Mompha*, Shaw (2012) considered that the species is probably monophagous, at least in Great Britain. Here it is recorded for the first time for the Nearctic, from Canada (MB, Churchill); *Mompha raschkiella* also occurs in Canada, but is not considered to be native there (J.F. Landry, personal communication). The difference between the two available haplotypes (one haplotype from several Canadian specimens, the other from one Scottish specimen) ranged between four and five base pairs (0.6–0.8%).

This species was described too late for inclusion in Yu et al. (2012). The reared material in NMS is all from the only known host, *Mompha raschkiella* (Det. K. P. Bland, E. S. Bradford, R. J. Heckford, E. C. Pelham-Clinton, M. R. Shaw, P. A. Sokoloff, M. R. Young).

11) *Microplitis kewleyi* Muesebeck, 1922

This species is widely distributed within the Nearctic. Here it is recorded for the first time in three additional Canadian provinces (NL, Corner Brook; NS, Kentville Research Station; Cape Breton Highlands National Park, MacIntosh Brook; and PEI, Harrington), and one state in the United States (NJ, Metuchen). Three species of Noctuidae have been recorded as hosts of the species (Yu et al. 2012).

We analyzed 38 barcode sequences available for this species (35 of them barcode compliant), representing 1 haplotype from Canada.



Figures 8–12. *Microgaster raschkiellae* (all photos from specimens from the United Kingdom part of the original description of the species) **8** Mesoscutum and scutellum **9** Metasoma in lateral view **10** Wings **11** Habitus, dorsal view **12** Hind leg (excluding coxa).

12) *Pholetesor bedelliae* (Viereck, 1911)

This species is widely distributed within the Nearctic, and is also reported from Finland (Hellén 1977), Peru (de Huiza 1995), and was introduced to Hawaii (Fullaway 1950). Here it is recorded for the first time from one additional province in Canada (SK, Grasslands National Park); and one province in the United States (AK, Anderson, Highway 3; Delta Junction; Sutton on Glen Highway). Around 20 host species within seven families of Lepidoptera have been recorded as hosts of the species (Yu et al. 2012).

We analyzed 66 barcode sequences available for this species (60 of them barcode compliant), representing seven haplotypes from Canada and the United States. The difference between haplotypes ranged between one and five base pairs (0.2–0.8%).

13) *Pholetesor masoni* Whitfield, 2006

This species is widely distributed within the Nearctic, and also reported from Chiapas, Mexico (Whitfield 2006). Here it is recorded for the first time from two additional provinces/territories in Canada (MB, Churchill, several specimens; 22 km S of Camperville, one specimen; and YT, Pelly Crossing, one specimen). The new records expand the known distribution of the species northwards. Only the genus *Bucculatrix* (Lepidoptera: Bucculatricidae) is known as host for this wasp species.

We analyzed eight barcode sequences available for this species (seven of them barcode compliant), representing four haplotypes from Canada. The difference between haplotypes ranged between one and seven base pairs (0.2–1.0%).

14) *Pholetesor rhygoplitoides* Whitfield, 2006

Previously known records for this species show a wide distribution within the Nearctic, although records are scarce and sparse over North America. Here it is recorded for the first time from two additional provinces in Canada (AB, Banff National Park, one specimen; and NL, Gros Morne National Park, Western Brook Pond, Hiking Trial, one specimen). No host is known for this species.

We analyzed three barcode sequences available for this species (two of them barcode compliant), representing one haplotype from Canada.

15) *Pholetesor salicifoliellae* (Mason, 1959)

This species is widely distributed within the Nearctic. Here it is recorded for the first time from two additional provinces in Canada (YT, Champagne; Takhini River road; and NT, Inuvik), and one province in the United States (AK, King Salmon, Naknek River; Nome). The new records expand the known distribution of the species northward. Around 16 host species of Gracillaridae and one species in Elachistidae (Lepidoptera) have been recorded as hosts of the species (Yu et al. 2012).

We analyzed 40 barcode sequences available for this species (39 of them barcode compliant), representing 13 haplotypes from Canada and the United States. The difference between haplotypes ranged between one and eight base pairs (0.2–1.2%).

16) *Pholetesor viminetorum* (Wesmael, 1837)

This species is widely distributed in the Holarctic (Yu et al. 2012). Here it is recorded for the first time from numerous specimens from four additional provinces/territories in Canada (NL, Cap St-George; Plum Point; Sally's Cove; South Branch; St-David's; NB, vicinity of Tracy; NT, Ford Lake; and SK, N of Rocanville, Qu'Appelle Road). More than 40 host species within 13 families of Lepidoptera have been recorded as hosts of the species (Yu et al. 2012), along with two records of Diptera (Agromyzidae and Tephritidae) which we consider to be almost certainly erroneous. The numerous reared specimens in NMS, all from species of *Elachista* (Lepidoptera: Elachistidae), are enumerated by Shaw (2012).

We analyzed 85 barcode sequences available for this species (76 of them barcode compliant), representing 28 haplotypes from Canada. The difference between haplotypes ranged between one and 12 base pairs (0.2–1.9%).

Acknowledgements

Teresa Martin did the lab work required to obtain the new sequence data. Agriculture and Agri-Food Canada provided funding for the sequencing of some specimens from the CNC and NMS. MRS is grateful to all those who donate reared specimens to him for the NMS collection. Jeremy deWaard, Jayme Sones, Paul Hebert, and Alex Smith kindly allowed JFT to access sequences and examine photographs of specimens collected by and deposited in the Biodiversity Institute of Ontario. Pelle Magnusson and his colleagues at the Swedish Museum of Natural History in Stockholm and the Station Linné in Öland (Sweden) sent specimens of Microgastrinae collected as part of the Swedish Malaise Trap Project. The reviews of Julia Sigenberg and an anonymous reviewer improved the final version of the manuscript.

References

- Achterberg C van (2012) Fauna Europaea: Microgastrinae. Fauna Europaea version 2.5, <http://www.faunaeur.org>
- De Saeger H (1944) Microgasterinae (Hymenoptera: Apocrita). Exploration du Parc National Albert. Mission G.F. de Witte. 47: 1–342.
- Fernández-Triana J, Goulet H, Bostanian N, Boudreault C (2009) Diversity of Microgastrinae (Hymenoptera: Braconidae) in apple orchards of southern Quebec, Canada. *Biocontrol Science and Technology* 19 (3): 237–248. doi: 10.1080/09583150802684406
- Fernández-Triana J (2010) Eight new species and an annotated checklist of Microgastrinae (Hymenoptera: Braconidae) from Canada and Alaska. *ZooKeys* 63: 1–53. doi: 10.3897/zookeys.63.565
- Fernández-Triana J, Smith MA, Boudreault C, Goulet H, Hebert PDN, Smith AC, Roughley R (2011) A poorly known high-latitude parasitoid wasp community: unexpected diversity and dramatic changes through time. *PLoS ONE* 6(8): e23719. doi: 10.1371/journal.pone.0023719

- Fernández-Triana J, Whitfield JB, Rodriguez JJ, Smith MA, Janzen DH, Hallwachs W, Hajibabaei M, Burns JM, Solis MA, Brown J, Cardinal S, Goulet H, Hebert PDN (2014) Review of *Apanteles* sensu stricto (Hymenoptera: Braconidae, Microgastrinae) from Area de Conservación Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. *Zookeys* 383: 1–565. doi: 10.3897/zookeys.383.6418
- Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology* 11: 839–850. doi: 10.1046/j.1365-294X.2002.01485.x
- Fullaway DT (1950) *Apanteles* in Hawaii. *Proceedings of the Hawaiian Academy of Sciences* 25: 7.
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270: 313–321. doi: 10.1098/rspb.2002.2218
- Hebert PDN, Ratnasingham S, DeWaard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society B* 270 (Suppl. 1): S96–S99. doi: 10.1098/rspb.2002.2218
- Hellén W (1977) För Finlands fauna nya *Apanteles*-arter (Hymenoptera, Braconidae). *Notulae Entomologicae* 57: 9–10.
- de Huiza R (1995) Diversidad de Braconidae (Hymenoptera) en el Peru. *Revista Peruana de Entomología* 37: 11–22.
- Ivanova NV, Dewaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6 (4): 998–1002. doi: 10.1111/j.1471-8286.2006.01428.x
- Janzen DH, Hallwachs W, Blandin P, Burns JM, Cadiou J, Chacon I, Dapkey T, Deans AR, Epstein ME, Espinoza B, Franclemont JG, Haber WA, Hajibabaei M, Hall JPW, Hebert PDN, Gauld ID, Harvey DJ, Hausmann A, Kitching I, Lafontaine D, Landry J, Lemaire C, Miller JY, Miller JS, Miller L, Miller SE, Montero J, Munroe E, Rab Green S, Ratnasingham S, Rawlins JE, Robbins RK, Rodriguez JJ, Rougerie R, Sharkey MJ, Smith MA, Solis MA, Sullivan JB, Thiaucourt P, Wahl DB, Weller SJ, Whitfield JB, Willmott KR, Wood DM, Woodley NE, Wilson JJ (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources* 9 (Supplement 1): 1–26. doi: 10.1111/j.1755-0998.2009.02628.x
- Mason WRM (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. doi: 10.4039/entm113115fv
- O'Hara J, Shima H, Zhang C (2009) Annotated Catalogue of the Tachinidae (Insecta: Diptera) of China. *Zootaxa* 2190: 1–236.
- Papp J (1988) A survey of the European species of *Apanteles* Först. (Hymenoptera, Braconidae: Microgastrinae). 11. “Homologization” of the species-groups of *Apanteles* s.l. with Mason's generic taxa. Checklist of genera. Parasitoid/host list 1. *Annales Historico-Naturales Musei Nationalis Hungarici* 80: 145–175.
- Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364. doi: 10.1111/j.1471-8286.2007.01678.x
- Rodriguez JJ, Fernández-Triana J, Smith MA, Janzen DH, Hallwachs W, Erwin TL, Whitfield JB (2012) Extrapolations from field studies and known faunas converge on dramatically

- increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). *Insect Conservation and Diversity* 6: 530–536. doi: 10.1111/icad.12003
- Shaw MR (2009) *Cotesia* Cameron (Hymenoptera: Braconidae: Microgastrinae) parasitoids of Heliconiinae (Lepidoptera: Nymphalidae) in Europe, with description of three new species. *British Journal of Entomology and Natural History* 22: 133–146.
- Shaw MR (2012) Notes on some European Microgastrinae (Hymenoptera: Braconidae) in the National Museums of Scotland, with twenty species new to Britain, new host data, taxonomic changes and remarks, and descriptions of two new species of *Microgaster* Latreille. *Entomologist's Gazette* 63: 173–201.
- Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN (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
- Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PDN (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 MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, Hallwachs W, Hebert PDN (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 MA, Fernández-Triana JL, Eveleigh E, Gómez J, Guclu C, Hallwachs W, Hebert PDN, Hrcék J, Huber JT, Janzen DH, Mason PG, Miller SE, Quicke DLJ, Rodriguez JJ, Rougerie R, Shaw MR, Varkonyi G, Ward D, Whitfield JB, Zaldívar-Riverón A (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
- Stahlhut JK, Fernández-Triana J, Adamowicz SJ, Buck M, Goulet H, Hebert PDN, Huber JT, Merilo M, Sheffield CS, Woodcock T, Smith MA (2013) Diversity of Hymenoptera and the dominance of parasitoids in a sub-Arctic environment. *BMC Ecology* 13: 2. doi: 10.1186/1472-6785-13-2
- Whitfield JB (1995) Checklist of the Microgastrinae (Hymenoptera: Braconidae) in America north of Mexico. *Journal of the Kansas Entomological Society* 68: 245–262.
- Whitfield JB (1997) Subfamily Microgastrinae. In: Wharton RA, Marsh PM, Sharkey MJ (Eds). *Manual of the New World genera of the family Braconidae* (Hymenoptera). Special Publication No. 1 International Society of Hymenopterists, Washington, D.C.: 333–364.
- Whitfield JB (2006) Revision of the Nearctic species of the genus *Pholetesor* Mason (Hymenoptera: Braconidae). *Zootaxa* 1144: 1–94.
- Williamson GD (1963) Summary of parasite and predator liberations in Canada and of insect shipments from Canada in 1963. *Canadian Insect Pest Review* 41: 137–151.
- Yu DSK, van Achterberg C, Horstmann K (2012) Taxapad 2012, Ichneumonoidea 2011. Ottawa, Ontario, Canada. Database on flash-drive. www.taxapad.com