Rearings of four European Microgastrinae (Hymenoptera: Braconidae), three new to Britain including a new species of *Cotesia* Cameron, 1891

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Abstract

Cotesia carterocephali sp. nov. is described from a single gregarious brood reared from a posthibernation final instar larva of the hesperiid butterfly *Carterocephalus palaemon* in Scotland. Details of English rearings of two other Microgastrinae recorded from Britain for the first time are also given: *Distatrix pompelon*, gregariously from the erebiid lymantriine *Orgyia antiqua* (also recorded from the same host in Austria), and *Dolichogenidea hemerobiellicida*, solitarily from the coleophorid *Coleophora hemerobiella* (with a record from possibly the same host in the Netherlands). The non-British *Glyptapanteles aletta* is recorded as a solitary parasitoid of *Limenitis populi* in Finland, France and Germany: a previous host record for this parasitoid is regarded as incorrect.

Key words: Carterocephalus, Coleophora, Limenitis, Orgyia, Cotesia carterocephali sp. nov., Distatrix pompelon, Dolichogenidea hemerobiellicida, Glyptapanteles aletta, biology, phenology, Austria, Finland, France, Germany, Netherlands

Introduction

The large and important braconid subfamily Microgastrinae exclusively parasitizes Lepidoptera, and globally there are well over 3000 described species in 81 genera (Fernandez-Triana et al., 2020; many more species have been described since that paper was published, though only one from Britain). An undoubtedly huge number of species await formal description. Even the British fauna is very incompletely known, despite great efforts by British workers D. S. Wilkinson and his field-worker collaborator R. L. E. Ford and subsequently G. E. J. Nixon in the middle part of the last century, which extensively reshaped knowledge of the group with foundation work that preceded the global generic revision of Mason (1981). New discoveries in Britain of existing species as well as new species have been of regular occurrence since the first modern checklist by Fitton et al. (1978): the most recent checklist (Broad, Shaw & Godfray, 2016) references the 26 species of Microgastrinae added to the British fauna since Fitton et al. (1978), with two further species added since then (Shaw & Fernandez-Triana, 2020; Shaw, 2021). Frequent additions are likely to continue, especially if lepidopterists make the effort to ensure that the parasitoids they accidentally rear are preserved and reach the researchers who can make the most of them.

Reared specimens of three species new to Britain that have recently come to light are outlined below, including description of a new species of *Cotesia* Cameron. In addition, the first reliable rearing records of another European (but non-British) species are presented. Examples of all four species are deposited in the National Museums of Scotland (NMS).

Methods

Terminology mostly follows van Achterberg & Shaw (2016), with some terms commonly used for Microgastrinae following Nixon (1965). Photographs were taken in RAW down one arm of a Wild 5A binocular microscope with $20 \times$ eyepieces using a Canon PowerShot S110 and processed using Adobe Photoshop Elements.

DNA barcodes were obtained from two paratypes, using the standard animal locus of the 5' region of the cytochrome c oxidase I (COI) gene (Hebert *et al.*, 2003). Briefly, DNA extracts were obtained from single legs using a glass fibre protocol (Ivanova, deWaard & Hebert, 2006), and total genomic DNA was resuspended in 30 μ l of dH2O. The barcode region, 658 base pairs (bp) region near the 5' terminus of the COI gene, was amplified using standard primers following established protocols. All information for the sequences associated with each individual specimen barcoded (including primers and trace files) is available on the Barcode of Life Data System (BOLD) (https://www.boldsystems.org/).

Description of new species

Cotesia carterocephali Shaw sp. nov.

(Figs 1–15)

MATERIAL EXAMINED

A single brood of $2\degree$, 2𝔅 (with 5 emerged cocoons). Holotype \degree 'SCOTLAND: Argyll, Glasdrum Wood 2.iv.2015 coll. P. Eeles ex Carterocephalus palaemon final inst. larv., 1 of 5 (4 pres.)', in the Natural History Museum, London (NMHUK). Paratypes $1\degree$, 2𝔅, same data as holotype, 1𝔅 in NMHUK, $1\degree$, 1𝔅 in NMS.

Diagnosis. Dark and rather slender; head only moderately transverse, evenly rounded behind eves; mesonotum rather strongly (rugo)punctate posteriorly and somewhat matt, scutellum more shiny with distinct punctures anteriorly; fore wing with membrane moderately infumate and dark setae rather evenly distributed in basal and subbasal cells, pterostigma uniformly dark, metacarp about 4 times as long as its distance from apex of radial cell, radius issuing from rather slender pterostigma at about distal 0.6; vannal lobe of hind wing with distinct fringe of setae; no spine near apex of front tarsal segment 5; hind leg with femur practically completely black, tibia reddish yellow but strongly infuscate in apical half, spurs unequal, inner one reaching past middle of basitarsus; T1 parallel sided, longer than wide, T2 with basal field occupying practically the whole tergite, transverse, moderately rugose and about 0.75 as long as T3, T3 shining, unsculptured, with setae sparse and distributed largely in a single subposterior row; hypopygium modest in size, with long setae, 0.55 as long as hind tibia, scarcely truncate but roundly descending to about 90° apically; ovipositor sheath moderately protruding. Gregarious parasitoid of Carterocephalus palaemon (Pallas, 1771) (Hesperiidae).

This species almost immediately runs into difficulty in Nixon's (1974) key to the 'Apanteles glomeratus-group' (now Cotesia) because (on account of the rather strong rugo-punctation of the mesoscutum and punctate scutellum) at couplet 2 it may run best to couplet 3, then (on account of its long hind tibial spurs) to couplet 4 covering C. callimone (Nixon) and C. setebis (Nixon). It differs from C. callimone in several respects including its longer metacarp, its shinier hind coxa, face, temple and occiput, as well as darker leg colour and in its unsculptured, strongly shining and weakly setose T3. [It should be noted that Nixon's (1974) concept of C. callimone included two species (Shaw, 2009)]. From C. setebis, the type series (examined) of which might well also contain more than one species, it differs most obviously in its less prominent hypopygium and (especially from the holotype) in its much more strongly sculptured mesoscutum and scutellum. If it is run the other way in Nixon's (1974) troublesome couplet 2, which might be more appropriate as the anterior of the mesoscutum is much less clearly punctured than posteriorly, it runs quite smoothly to couplet 38 separating C. eurvale (Nixon) from C. zvgaenarum (Marshall). From Nixon's (1974) description of the former it differs in having distinctly though weakly infumate wings with rather uniformly coloured venation, ocelli in a higher triangle, scutellum quite sharply punctured, T1 not widening posteriorly, T3 only sparsely setose, etc. From C. zvgaenarum it differs in the lesser degree of stark contrast between the black hind femur and its tibia (almost entirely vellow in C. zvgaenarum), its darker wing membrane, longer metacarp, radius leaving the narrower pterostigma more distally, less pronounced hypopygium, etc. Another dark Cotesia that parasitizes grass-feeding butterflies (in this case Satyrinae) is C. tetrica (Reinhard), but the new species differs in many respects, including its longer metacarp, less widened T1, less transverse T2 and T3, less sculptured T3 (i.e. T2+3 posterior to the basal field), longer hind tibial spurs, shinier mesoscutum and (especially) scutellum, etc.

In Papp's (1986) key to species groups of [Cotesia] the new species runs to the glomerata-subgroup and in Papp's (1987) key to that species group rather easily to couplet 126, also ending with C. euryale and C. zygaenarum. Papp (1990) published again on Cotesia, but the few additional species dealt with do not suggest a possible placement. In the 1995 translation of Kotenko & Tobias's (1986) key it cannot be run to any of the species dealt with between couplets 120 and 359 (essentially Cotesia) with any confidence, and indeed it runs most easily to species with which, based on other information, it invariably disagrees.

Description

Holotype \mathcal{Q} . Length 2.5 mm, of fore wing 2.9 mm.

Head in dorsal view $1.7 \times$ as wide as long, temple $0.9 \times$ as long as eye and evenly rounded; ocelli in moderately high triangle, tangent to posterior pair not cutting anterior ocellus; OOL 2.3x and POL 2x diameter of a posterior ocellus; vertex, stemmaticum and frons weakly sculptured, shiny; face (excluding clypeus) $1.1 \times$ as wide as high, ridge marking junction of swollen areas below antennal insertions and extending onto face rather prominent; eves slightly diverging below; anterior tentorial pits deep, just below lower level of eves; face moderately shiny, shallowly rugose-punctate (clypeus less so); malar space 1.3× basal width of mandible; antenna 0.9x as long as fore wing, preapical segment 1.7x as long as wide. Mesosoma with mesoscutum anteriorly and on side lobes matt and rather finely rugulose-punctate, but centrally and posteriorly more shiny with larger punctures clearly defined; notaulic courses only weakly differentiated; pre-scutellar groove deep with 8-10 strong fovea; scutellum shiny, discretely punctured anteriorly, less evidently posteriorly; phragma of scutellum concealed by post-scutellum; mesopleuron with deep and rather wide precoxal area with rugae, a large smooth and shiny area anterior to/above it, and its anterior aspect matt and rugulose-punctate; propodeum moderately rugose, median carina evident but rather weak (some variation in the two females available, Figs 11, 12). Fore wing with basal and subbasal cells almost evenly and moderately densely setose; pterostigma narrow, 2.7× longer than wide, emitting radius subvertically at 0.6 its length; r as long as width of pterostigma and angled with 2RS with no stub; metacarp $1.1 \times$ as long as pterostigma and about $4.0 \times$ its distance from apex of marginal



Figs 1–8. *Cotesia carterocephali* sp. nov., female. Holotype (8); paratype (1–7). 1, habitus; 2, head in dorsal view; 3, face; 4, malar space; 5, flagellum; 6, hypopygium, ovipositor sheath, hind femur, most of middle leg; 7, wings; 8, hind wing vannal lobe.



Figs 9–15. Cotesia carterocephali sp. nov. Female (9–14); cocoons (15). Holotype (9, 12); paratype (10, 11, 13, 14). 9, hind tibia and spurs; 10, mesoscutum and scutellum; 11, propodeum and hind coxae in dorsal view; 12, propdeum; 13, second and third metasomal tergites; 14, first metasomal tergite; 15, cocoons.

cell. Hind wing vannal lobe with distinct hair fringe. Hind leg with coxa almost smooth and moderately shiny on outer side, dorsally rugulose; femur $4.0 \times$ as long as wide; inner tibial spur longer than outer and reaching just beyond middle of basitarsus. Fore leg lacking spine on distal tarsal segment. Metasoma with T1 roundly arched in lateral view, $1.2 \times$ longer than wide, parallel-sided, rugose-punctulate and partly shiny centrally/posteriorly; T2 with rugose rectangular basal field occupying practically whole tergite, $2.3 \times$ wider than long, with crenulate posterior margin, $0.75 \times$ as long as T3; T3 $1.9 \times$ wider than long, smooth and shiny except at extreme anterior edge, setae largely confined to a sparse single row sub-posteriorly with a few post-anteriorly at sides; hypopygium rather densely long-setose, $0.55 \times$ as long as hind tibia, scarcely truncate but roundly attenuating at just under 90° in lateral view; ovipositor sheath shortly protruding, slightly downcurved and dagger-shaped.

Colour: black. Palpi and the following parts of legs reddish or brownish yellow: front leg including and beyond trochantellus; mid leg trochantellus, femur below and apically, tibia and tarsus; hind leg trochantellus, proximal two thirds of tibia, spurs and proximal part of basitarsus. Setae of wing membrane and most of venation including pterostigma dark.

Male. like female except for sexual characters.

Cocoons off-white, weakly connected, formed in the host retreat.

Peter Eeles (pers. comm.), whose fieldwork (Eeles, 2016) at Glasdrum Wood NNR and other sites was much inspired by that of Ravenscroft (1992), has provided the following further information. The parasitized host was one of eight followed through to their hibernacula as fully fed larvae. In this case, the host larva had been under observation in its resting site from October 2014 before the parasitoids erupted in spring and made the cocoons that were collected in situ on 2.iv.2015 so, although the host remains were not recovered, there is no doubt as to the identity of the host. The adults emerged (indoors) a week or two later. The other seven hosts were not parasitized. The phenology is consistent with this parasitoid being a specialist capable of completing its annual cycle with no other host required.

The rearing is mentioned in Toro-Delgado *et al.* (2022) and appears to be the only known instance of a *Cotesia* species parasitizing a member of the hesperiid subfamily Heteropterinae in Europe. There seems to be no record of *Cotesia* parasitoids of European Hesperiinae either, although Pyrginae are heavily parasitized by specialised *Cotesia* species (Toro-Delgado *et al.*, 2022).

The two male paratypes have been barcoded and the CO1 sequences are available in BOLD with voucher codes (referred to as 'sampleID' in BOLD) MRS_JFT0716 and MRS_JFT0717.

Other species new to Britain

Distatrix pompelon (Nixon, 1965)

(Fig. 16)

 1° , 1° ex *Orgyia antiqua* (Linnaeus, 1758) collected in its final instar on 17.viii.2021 on *Quercus* at Rowney Warren plantation, Bedfordshire, TL192402 (R. Revels). The adults emerged around 6.ix.2021 from a brood of about a dozen white cocoons constructed by about 27.viii.2021 in loose association around and under the host. These dates are approximate: Richard Revels (pers. comm.) observed the host larva to feed a little for a few days then remain quiescent for about a week before the parasitoid larvae erupted and made their cocoons, and the two adults had emerged about a week before he sent them to me for determination on 13.ix.2021. They are now in NMS, including cocoons and host remains. One is barcoded and the CO1 sequence is available in BOLD referenced as MRS_JFT0950.

In addition to the above, part of a brood of $5\,$ °, $2\,$ ° of *D. pompelon* reared from *O. antiqua* (host remains examined by MRS) in Austria (Oberösterreich: Aschachtal) in 2019 (M. Schwarz), was received for determination. Specimens of both sexes are now in NMS, and also (with the host remains) in Biologiezentrum, Linz. One in NMS has been selected for attempted barcoding (not yet completed) and is referenced in BOLD as MRS_JFT0836.

Nixon (1965) described this parasitoid (as *Apanteles*) from a single specimen reared in Japan from *Euproctis similis* (Fuessly, 1775), and later (Nixon, 1973) mentioned a specimen determined by him and published by Čapek (1972) that had been reared from the same host in what has become Slovakia. The detail that the propodeal spiracle interrupts the bordering lateral keel of the propodeum in the Japanese holotype cited by Nixon in 1965 was not repeated in his 1973 account, and in all the specimens in NMS the spiracle, although close to the bordering keel, remains discrete and does not disrupt it. In concert with a check on generic placement in Mason (1981), noting especially the very few and almost invisible setae of the ovipositor sheath, *Distatrix pompelon* (as *Apanteles pompelon*) would be identifiable through Nixon's 1965 and 1973 papers (in which he includes it in his '*Apanteles formosus*-group'). The smooth and strongly transverse triangular basal field of T2, the pale anterior part (at least) of T1, and the lateral compression of the posterior part of the metasoma are useful supporting characters.

Since *E. similis* overwinters as a small larva and *O. antiqua* as eggs, the rearings from these two lymantriines suggest that *D. pompelon* may be plurivoltine, with *O. antiqua* serving as host for a summer brood and *E. similis* allowing the parasitoid to overwinter within a diapausing host. The rearings presented here show it to be gregarious.

Dolichogenidea hemerobiellicida (Fischer, 1966) (Fig. 17)

 1° ex *Coleophora hemerobiella* (Scopoli, 1763) (Coleophoridae) case collected at Oxford, 21.iv.2017 (B. Henwood). The case is present but there is no emergence date. The material is in NMS, and agrees closely with a female paratype deposited in NHMUK.

Also in NMS is 1δ with data: Holland, Malden, Heumensoord ex *Coleophora* sp. on whitebeam collected 13.vii.1977, emerged 31.vii.1977 (K. P. Bland). It agrees well with a male paratype in NHMUK. The case is present and appears to be compatible with *C. hemerobiella*.

Fischer (1966) described *Dolichogenidea hemerobiellicida* (as *Apanteles*) from 2, 4δ reared solitarily from *Coleophora hemereobiella* in Austria, and it is included in keys by Papp (1980) and Kotenko & Tobias (1986, translated 1995). It is a moderately distinctive species, the female having the fore wing pterostigma practically uniform brown; all femora, tibiae and tarsi orange; ovipositor sheath about half the length of the hind tibia, with the ovipositor itself rather evenly but not strongly downcurved; T1 distinctly narrowing towards apex (more so than in other species of *Dolichogenidea* with more or less yellow legs that parasitise arboreal *Coleophora* species, such as *D. breviventris* (Ratzeburg) which anyway has the pterostigma with a distinct proximal yellow spot and a longer ovipositor); mesoscutum shiny and sharply punctured; antenna with penultimate segment about 1.8× longer than wide. In the male the pterostigma is paler yellowish, and the legs are darker than in the female. It is not treated by Nixon in either of his



Figs 16-18. 16, Distatrix pompelon, basal metasomal tergites; 17, Dolichogenidea hemerobiellicida, alitrunk in dorsal view; 18, Glyptapanteles aletta, basal antennal segments.

keys that cover the majority of species now included in *Dolichogenidea*, as the '*Apanteles laevigatus*-group' (Nixon, 1972), and the '*Apanteles metacarpalis*group'(Nixon, 1973), and in these keys it runs best, though not very smoothly, to species (respectively *D. phaola* (Nixon) and *D. ate* (Nixon)) that have black hind femora (and several other differences).

How *D. hemerobiellicida* adapts to the biennial life cycle of its host is unclear, but it seems quite likely to be a monophagous parasitoid.

The English specimen is barcoded and the CO1 sequence is available in BOLD referenced as MRS-JFT 0852.

A further reared species from Europe

Glyptapanteles aletta (Nixon, 1973) (Fig. 18)

There are six females and one male of this species in NMS, all reared solitarily from part-grown larvae of *Limenitis populi* (Linnaeus, 1758) (Nymphalidae, Limenitidinae). All are accompanied by the light pinkish buff cocoon, and several by the host remains. The data are:

3 $\[mathbb{Q}$ S. E. Finland, Parikhala, ex *L. populi* on *Populus tremula*, ex 3rd instar, em. 4.vi.1984, 6.vi.1984 and 12.vi.1984 (P. Kantonen); 1 $\[mathbb{Q}$, same data except instar not recorded, coll. 9.viii.1983, cocoon formed 1983, emergence date not recorded; 1 $\[mathbb{Q}$, France, Haute-Savoie, coll. as cocoon with moribund *L. populi* larva 26.vi.2019, em. 4.vii.2019 (Y. & J.-L. Pelouard); 1 $\[mathbb{Q}$, France, Alpes-de-Haute-Provence, coll. as cocoon with moribund 4th instar *L. populi* larva 26.vi.2021, em. 9.vii.2021 (Y. & J.-L. Pelouard) [another cocoon with same data and L4 host remains failed to emerge]; 1 $\[mathbb{S}$, Germany, Bavaria, Forchheim ex *L. populi* coll. 1986, cocoon formed 20.v.1987, em. 29.v.1987 (H. G. Short) [accompanied by ?L4 host remains].

The rearing data make it clear that the parasitoid overwinters in the small overwintering larva of this strictly univoltine host, killing it well before it attains full growth in the following summer. It is also evident that there can be a further annual brood, perhaps only partial (though the possibility that the parasitoid habitually has two broods successively on a single host generation cannot be ruled out), erupting from early instar pre-hibernation larvae (the Finnish individual whose host was collected on 9.viii.1983). In this case the adult parasitoids would presumably hatch quickly in time to parasitise the same host generation before it entered hibernation. The single specimen with this apparent phenology made its cocoon on the terminal part of the midrib of a *P. tremula* leaf exposed by the feeding of the early instar host larva; the other cocoons all appear to have been formed on narrow twigs. No other host species is necessary for the parasitoid to complete its annual cycle.

Nixon (1973) described this species (as *Apanteles*) from two non-reared specimens from Finland. It can be determined from his 1973 key to the '*Apanteles vitripennis*-group' provided it is realised that the long pubescence of the flagellum is well-marked only on the proximal segments, and then largely on one side (Fig. 18). There are two apparent rearing record of this species citing the choreutid *Choreutis pariana* (Clerck, 1759) (Yu, van Achterberg & Horstmann, 2016). The first is by Kotenko & Tobias (1986), and the host is given a question mark (not transferred to Yu, van Achterberg & Horstmann, 2016); the second (Čapek & Hofmann, 1997) is just a reiteration (without the source identified, and again without the question mark); an unfortunate practice without scientific merit (Shaw, 2017). In any case, *Choreutis pariana* is not considered to be a reliable host record, and the rearings from *L. populi* given here are believed to represent the true, and probably only, host.

Two French specimens have been barcoded and the CO1 sequences are available in BOLD referenced as MRS_JFT0952, MRS_JFT0953. A third specimen, also French, referenced as MRS_JFT0827 has been selected for barcoding but has not yet been sequenced.

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