Two new European species of *Cotesia* Cameron, 1891 (Hymenoptera: Braconidae, Microgastrinae) parasitizing butterflies (Lepidoptera: Papilionoidea), and an unrelated synonymy in the genus

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Abstract

Two new European species of *Cotesia* are described and illustrated. One (*C. euchloevora* Shaw, sp. nov.) was reared from *Euchloe* species and close relatives and also *Aplocera efformata*, and the other (*C. parnassii* Shaw, sp. nov.) was reared from *Parnassius phoebus*. Notes are given to distinguish them from congeners. The DNA barcodes of *C. euchloevora* are discussed in relation to similar barcodes for another, but morphologically clearly distinct, species *Cotesia pilicornis* (Thomson). Unrelated to this, it is proposed that *C. acutula* (Tobias) is a junior synonym of *C. cajae* (Bouché).

Keywords: *Aplocera*, *Euchloe*, *Zegris*, *Parnassius*, *Cotesia euchloevora* sp. nov., *Cotesia parnassii* sp. nov., *Cotesia pilicornis*, *Cotesia cajae*, *Cotesia acutula*, biology

Introduction

The microgastrine braconid genus *Cotesia* includes about 328 species currently regarded as valid, out of an estimated world total of around 1500–2000 species (Fernandez-Triana *et al.*, 2020). In such a large genus, of sometimes very closely similar species, it is relevant to consider what useful purpose will be served when deciding whether or not to generate one-off descriptions of new species from specimens defying identification, as there is a risk (already materialising) of simply cluttering the classification with problems for future workers. However, when an apparently undescribed species has associated biological information it is more certainly appropriate to describe it, including a diagnosis to separate it from closely similar species, in order to pin biological knowledge to a name, and this principle guides us here.

Cotesia species are among the most important parasitoids of butterflies in Europe (Shaw, Stefanescu & Nouhuys, 2009), and many such species are known, usually exhibiting at least moderate levels of specialisation and in many cases being extreme specialists. In the present work two additional *Cotesia* species parasitizing European butterflies are described, the first reared from *Euchloe* species and the closely related *Zegris eupheme* (Esper) (Pieridae) as well as the unrelated *Aplocera efformata* Guenée (Geometridae), and the second from *Parnassius phoebus* (Fabricius) (Papilionidae). Additionally, a synonym of another *Cotesia* species (neither a parasitoid of butterflies, nor close to either of the above) is proposed.

Methods

Morphological terminology largely follows van Achterberg & Shaw (2016), with the addition of some terms traditionally applied to Microgastrinae in particular, such as metacarp, vannal lobe, and phragma of scutellum, which are used in the sense of Nixon (1965) and his subsequent papers. For further clarity, POL (posterior ocellar line) is the distance between the posterior ocelli, OOL (ocular ocellar line) is the shortest distance between the eye and posterior ocellus, and malar space is the shortest distance between the eye and articulation of the mandibular condyle.

Photographs were taken down one arm of a Wild M5A binocular microscope with ×20 eyepieces using a Canon PowerShot S110.

Depositories:
CNC = Canadian National Collection of insects, Ottawa
HNHM = Hungarian Natural History Museum, Budapest
NHMUK = Natural History Museum, London
NMS = National Museums of Scotland, Edinburgh
RMNH = Naturalis Biodiversity Centre, Leiden
ZIN = Zoological Institute, St Petersburg

Barcodes were obtained using DNA extracts from single legs using a glass fibre protocol (Ivanova, deWaard & Hebert, 2006). Extracts were re-suspended in 30μl of dH_2O, and a 658-bp region near the 5' terminus of the CO1 gene was amplified using standard primers (LepF1–LepR1) following established protocols (Smith *et al.*, 2006). All information for the sequences associated with each specimen barcoded can be retrieved from the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007).

For phylogenetic analysis, 17 sequences from nine *Cotesia* species (including seven sequences from one of the species described below, and three from the taxon closest to it according to BOLD) were aligned using the default settings for Muscle in MEGA X (Kumar *et al.*, 2018). The final aligned dataset contained 658 characters. To explore phylogenetic relationships between this new species and other *Cotesia* species, evolutionary analyses were conducted in MEGA X, using the Maximum Likelihood method and Tamura-Nei model. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories ($+G$, parameter $= 0.6469$)). The rate variation model allowed for some sites to be evolutionarily invariable ([+/], 39.70% sites). The tree with the highest log likelihood was selected and drawn to scale, with branch lengths measured in the number of substitutions per site.

Description of new species

Cotesia euchloevora Shaw sp. nov.
(Figs 1, 2; habitus Fig. 1A)

Diagnosis. The new species falls into a group of solitary parasitoids having antennae with the preapical segments short, the ocelli in a low triangle, the

phragma of the scutellum narrowly visible, the pterostigma in the fore wing short and broad (about $1.8 \times$ as long as broad), the vannal lobe of the hind wing with indistinct or no fringe of setae, the hind femur black and the hind tibial spurs (sub)equal. In Nixon's (1974) key it would run to [*Cotesia*] *numen* (Nixon), sharing many characters (in addition to the above, the even distribution of setae in the basal and subbasal cells, length of metacarp (1-R1), lack of spine on the fore telotarsus, and general sculpture) but it differs from that species in having the eyes more converging below leading to a narrower face, the second metasomal tergite more transverse and the third with less tendency to be sculptured, more hyaline wings with the base of the metacarp contrastingly yellowish where it leaves the dark pterostigma, and the proximal internal venation and setae colourless, and (usually) slightly shorter preapical antennal segments. In the shape of its face it approaches [*Cotesia*] *praepotens* sensu (Nixon) not (Haliday), but differs from that species (which was judged by van Achterberg (1997) to be correctly *Cotesia brachycera* (Thomson)) in several characters including having longer antennae and preapical segments (preapical segments often transverse in *brachycera*), a less slender build (and lacking the rather pronounced posterior lateral compression of the metasoma of *brachycera*), denser and more evenly distributed setae in the basal cell of the fore wing (largely absent near M+CU1 in *brachycera*), the second metasomal tergite more transverse and the third tergite more densely setose. A further species described by Nixon (1974), [*C.*] *euryale*, shares *Aplocera* as host with the new species, but is not closely similar. Other available keys lack supporting information making them more difficult to use, but in that by Kotenko & Tobias (1986) it would run to [*C.*] *numen*, while in Papp's work (1986, 1987, 1990) it might run to [*C.*] *kazak* (Telenga), from which it differs in several respects including its less elongate build, shorter/broader pterostigma, and shorter and more truncate hypopygium. *Cotesia lineola* (Curtis) is another species with a short/broad pterostigma and short antennae which bears some resemblance to the new species and moreover, like it, has a host (the crambid *Evergestis forficalis* (Linnaeus)) feeding on Brassicaceae. However, *C. lineola* is a smaller and gregarious species, with generally weaker sculpture, eyes not markedly converging below, fore wing with membrane not at all hyaline and short metacarp (only about $2 \times$ as long as its distance from apex of marginal cell).

Taken together, the stark contrast between the hyaline fore wing membrane and the dark short/broad pterostigma, the colourless proximal venation and setae that are fairly evenly distributed in the basal cell, the pale base of the metacarp, the short preapical antennal segments, the convergence of the eyes below, and the black hind femur make this a fairly distinctive species.

We have seen the types of *C. numen*, *C. kazak* and *C. euryale*, the series treated by Nixon (1974) as [*C.*] *praepotens*, and also much material reared from the recorded hosts of these species and *C. lineola* in NMS and NHMUK. The conclusion that *C. brachycera* is the correct name for the species Nixon (1974) and subsequent authors before van Achterberg (1997) called [*C.*] *praepotens* is discussed at length by Fernandez-Triana *et al.* (2020).

MATERIAL EXAMINED

♀Holotype 'SPAIN: Granada, Baza. Ex Euchloe crameri, Moricandia moricandioides, A. González Megías 27.4.2011, coc. 2.5.11, em. 11.5.2011 72' (in National Museums of Scotland, Edinburgh). Paratypes (54♀, 41♂) as follows: 47♀, 27♂ with similar data to holotype, including host *Euchloe crameri* (Butler), on the plants (in order of frequency)
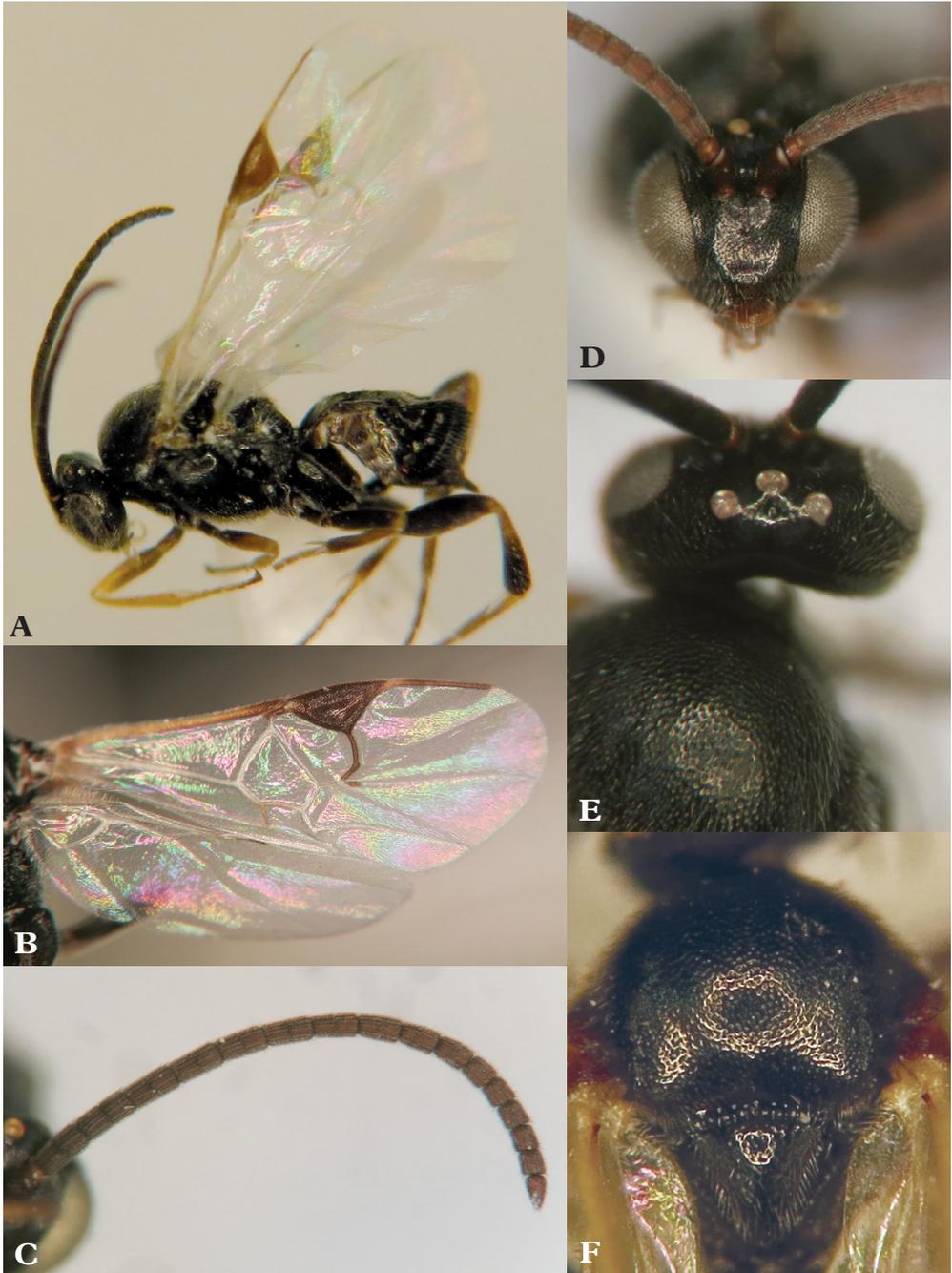
Fig. 1. *Cotesia euchloevora* sp. nov. Paratypes (all ex *Euchloe crameri*). **A**, habitus, lateral; **B**, wings; **C**, antenna; **D**, face; **E**, head, dorsal and mesoscutum, anterosublateral; **F**, mesoscutum and scutellum, dorsal.
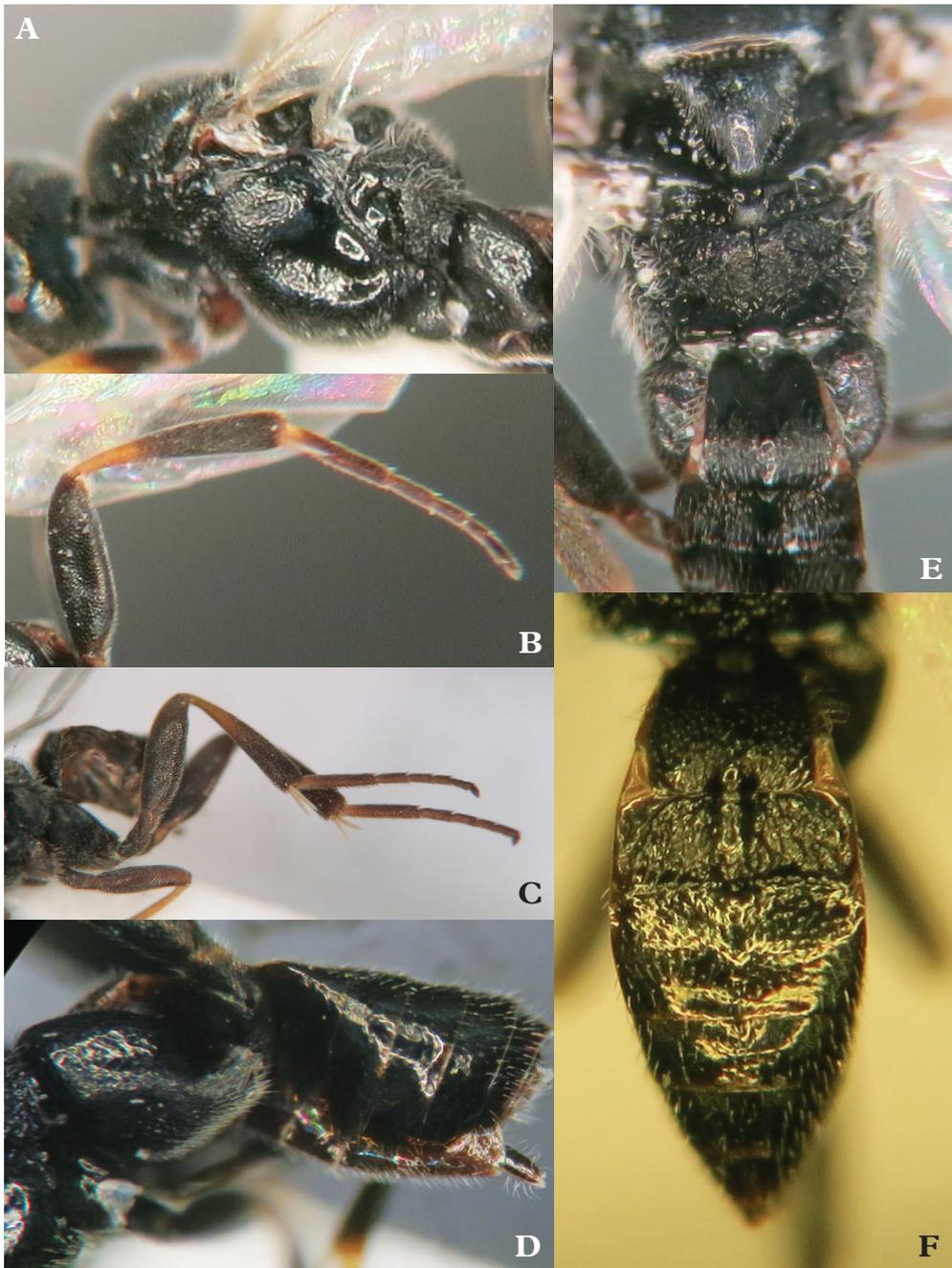
Fig. 2. *Cotesia euchloevora* sp. nov. Paratypes (all ex *Euchloe crameri*). **A**, mesosoma, lateral; **B**, **C**, hind leg, lateral; **D**, metasoma, lateral; **E**, scutellum, metanotum, propodeum and T1, dorsal; **F**, metasoma, dorsal.

Moricandia moricandioides, *Sisymbrium austriacum*, *Biscutella auriculata*, *Eruca vesicaria*, *Moricandia arvensis* or *Matthiola fruticulosa* with host collection dates in iv–v(–vi) and adult emergence dates in v–vi(–vii) in the years 2010, 2011, 2013 and 2016 (in NMS, CNC, HNHM, NHMUK, RMNH, ZIN); 1 ♀ with similar data to the foregoing but host *Euchloe belemia* (Esper) on *Sisymbrium* (NMS [DNA voucher MRS_JFT0600]); 3♂ with similar data to the foregoing but host *Zegris eupheme* on *Sisymbrium* (NMS [including DNA vouchers MRS_JFT0603 and MRS_JFT0595]); 1♀ Spain, Granada, Hoya de Baza, Benamaurel ex *E. crameri*, coll. 21.v.2001, em. 1.vi.2001, C. J. Luckens (NMS); 1♂ Spain, Badajoz, Fuente del Arco, ex *Euchloe tagis* (Hübner) on *Iberis ciliata contracta*, coll. 14.v.2014, em. 29.v.2014, R. Obregón (NMS [DNA voucher MRS_JFT0461]); 1♀ FRANCE Var, Callas, La Ferrage du Ray, ex *E. crameri*, v. 2013, P. & B. Kan (NMS [DNA voucher MRS_JFT0343] and 2♂ experimentally reared from it in *E. crameri*, vi.2013, P. & B. Kan (NMS); 3♀, 7♂ ENGLAND, Northumberland, Cambois, ex *Aplocera efformata* on *Hypericum*, coll. vi.2010, em. viii.2010, T. Tams & R. Leverton (NMS [including DNA voucher MRS_JFT0064]); 1♀ ?England, reared at Farnham House Laboratory, Surrey, ex *Aplocera efformata* or *plagiata* (Linnaeus), 16.vii.1935, S. Garthside (NHMUK); 1♂ NETHERLANDS, Gelderland, Hoge Veluwe National Park, ex *A. efformata* on *Hypericum*, coll. 15.vi.2015, coc. 21.vi.2015, em. 30.vi.2015, M. R. Shaw (NMS [DNA voucher MRS_JFT0572]). Most of the paratypes from Baza are slightly to rather badly faded owing to storage conditions.

Description

Holotype ♀. Length of body 2.4 mm, of fore wing 2.7 mm. Head in dorsal view (Fig. 1E) $2.0 \times$ as wide as long, widest across eyes, temple $0.8 \times$ as long as eye and strongly roundly narrowing behind eye. Ocelli in low triangle, tangent to posterior pair just cutting anterior ocellus, POL $2.5 \times$, OOL $1.9 \times$, and distance between anterior and posterior ocellus $0.8 \times$ posterior ocellar diameter, respectively. Frons shiny, vertex weakly setiferous-punctulate, moderately shiny. Face (Fig. 1D) $1.3 \times$ as wide as high (excluding clypeus), superficially rugulose-punctate and moderately shiny, eyes extending to just below upper margin of clypeus and obviously a little convergent at level of face; malar space about length of base of mandible. Antenna (Figs 1A, 1C) $0.7 \times$ as long as fore wing, segments (14)15–17 about as long as wide (ranging from cubic to $1.2 \times$ as long as wide in paratypes). Mesosoma (Fig. 2A) $1.4 \times$ as long as high. Mesoscutum (Figs 1E, 1F) rather evenly and densely setose, weakly but distinctly (rugulose-)punctate, the punctures more discrete posteriorly where it is most shiny, notaulic courses scarcely indicated; prescutellar suture (Fig. 1F) moderately deep with about 8–10 fovea; scutellum (Fig. 1F) rather raised, similarly sculptured as mesoscutum and shiny, evenly setose. Mesopleuron (Fig. 2A) anteriorly with discrete but rather weak punctures, precoxal area with weak crenulation but largely smooth and shiny, below this and mesosternum weakly rugulose-punctate. Phragma of scutellum slightly exposed (Fig. 2E). Propodeum (Fig. 2E) only moderately rugose with median longitudinal carina evident, the posterolateral more weakly sculptured area seeming somewhat depressed with strong bordering carinae. Metasoma (Figs 2E, 2F) with 1st tergite $1.3 \times$ as long as wide, gradually widening towards posterior where only slightly narrowed, apical turned over part rugose; 2nd tergite $3 \times$ as wide as long, basal field subtriangular and longitudinally rugose with distinct lateral sulci directed virtually to posterior corners (less so in some paratypes); 2nd tergite $0.6 \times$ as long as 3rd, the suture between them distinct; 3rd tergite essentially unsculptured and evenly setose; hypopygium (Fig. 2D) about $0.5 \times$ hind tibia, apically at about 80° and slightly truncate; ovipositor sheath weakly projecting. Fore wing (Fig. 1B) with pterostigma short and broad, $1.8 \times$ as long as wide and $0.9 \times$ metacarp, emitting radius (r) from about middle; metacarp $3.0 \times$ as long as its distance from apex of marginal cell; setae of basal cell evenly distributed. Hind wing (Fig. 1B) vannal lobe at and beyond widest part without fringe of projecting setae. Hind leg with coxa rather shiny with weak confused punctation (Figs 2A, 2D), femur (Fig. 2B) $3.3 \times$ as long as wide, tibia (Figs 2B, 2C) somewhat flattened and expanded apically with width near apex almost twice that at a third of its length (though variable) and spines on outer side (Fig. 1A) rather fine and even, tibial spurs (Fig. 2C) (sub)equal, not quite reaching midpoint of 1st tarsal segment. Fore leg without spine on telotarsus.

Colour. Black, mouthparts brown (palpi dirty whitish apically), hind and mid tibia in proximal half and fore leg distal to middle of femur brownish yellow, tegula and humeral plate sometimes and hind and mid tarsus mostly brownish (tarsi more yellowish in some paratypes), legs otherwise dark brown (hind femur black). Spurs rather pale. Wings decidedly hyaline, setae and internal venation proximal to pterostigma and areolet practically completely colourless (but 2-CU1 and cu-a light brown), metacarp brown but markedly yellowish near brown pterostigma, C+SC+R becoming yellow proximally.

Male. Similar to female except for sexual differences. Antenna 1.3× length of fore wing, attenuating to apex, penultimate segment about 3× as long as wide; sculpture tending to be weaker than in female.

   There is variation in intensity and clarity of both sculpture and colour; also in length of preapical antennal segments, and the ratio of lengths of 2nd and 3rd tergites. Cocoon (see below) varies in colour from pale yellowish to pinkish buff, and this can change with time.

Etymology. The specific epithet *euchloevora* is adjectival, meaning *Euchloe*-eating.

Molecular data (DNA barcoding). Several of the above specimens have been barcoded (Table 1); most rendered full barcode-sequences, though two (MRS_JFT0461 and MRS_JFT0595) gave shorter CO1 sequences of only 400+ base pairs. There is no doubt that the molecular evidence supports the morphological conclusion that the material identified here all belongs to one species, *Cotesia euchloevora* sp. nov. However (not accounting for the two shorter sequences), the species' sequences comprise two haplotypes (Fig. 5), differing by about 1% (6 base pairs), one in Spain (MRS_JFT0600 and MRS_JFT0603) and the other in France, England and the Netherlands (MRS_JFT0343, MRS_JFT0064 and MRS_JFT0572). The latter group comprises both *Cotesia* specimens reared from *Ap. efformata* and one reared from *E. crameri*, with identical CO1 sequences, while the Spanish group comprises two *Cotesia* sequences reared from close relatives of *E. crameri*, namely *E. belemia* and *Z. eupheme*, which are again identical. The approximately 1% difference between the two groups is here interpreted as intraspecific, perhaps reflecting the barrier to gene flow presented by the Pyrenees. The short sequences (MRS_JFT0595 and MRS_JFT0461) represent respectively another specimen reared from *Z. eupheme* (from the same Spanish site as the other one, MRS_JFT0603, from which site the vast bulk of the paratypes came) and one reared from *E. tagis* at a different site in Spain. Both shorter sequences cluster closest to the sequences from France, England and the Netherlands, with which they form a clade with 94% bootstrap support. It is practically inconceivable that there are two morphologically identical *Cotesia* species parasitizing *Z. eupheme* at the same site, to explain the distance between MRS_JFT0595 and MRS_JFT0603, but it is unfortunate that specimens reared from *E. crameri* from this main Spanish site (of which there are many) have not yet been barcoded, although specimens for that have been selected (MRS_JFT0593, MRS_JFT0596, MRS_JFT0599 and MRS_JFT0602) and it is hoped that molecular data from these will become available in the future.

   There is an additional complication that several sequences of *Cotesia* specimens identified as *C. pilicornis* (Thomson) available in BOLD cluster closely with two of the Spanish *C. euchloevora* sp. nov. (i.e. with MRS_JFT0600 and MRS_JFT0603), and three of these *C. pilicornis* specimens (reared from *Amblyptilia* species) are in NMS and so available for morphological verification (MRS_JFT0200, MRS_JFT0203 and MRS_JFT0208, see Table 1 and Fig. 5).

Despite being only one to three base pairs different from the Spanish group (0.1–0.4%) they, like many other series of *C. pilicornis* in NMS reared from a total of 11 species of Pterophoridae in the UK, Ireland, Finland, France and Spain, are clearly different morphologically from *C. euchloevora* sp. nov. Among several other differences, all females of *C. pilicornis* have the antenna at least slightly, sometimes much, more pubescent (there is intraspecific variation), a much more enlarged and acute hypopygium, and longer and generally broader ovipositor sheath. Both sexes of *C. pilicornis* have the pterostigma more normally shaped, about 2.0–2.1 × as long as broad (not as short/broad as in *C. euchloevora* sp. nov.), the metacarp uniformly coloured (not paler near pterostigma), and the face less narrowed below. It is clear that the examined specimens of *C. pilicornis* and *C. euchloevora* sp. nov. represent different species, despite the similarity in their barcodes. Whether or not this similarity is a result of stochastic convergence or reveals a close phylogenetic relationship is unclear, but in general barcodes are more useful for separating cryptic (morphologically very close) species than for reliably inferring relationships between morphologically divergent ones.

Biology. *Cotesia euchloevora* sp. nov. is a rather frequent solitary parasitoid of *Euchloe* species and closely related Pieridae in the tribe Anthocharini feeding on various Brassicaceae in the western Mediterranean region. The parasitoid larva erupts from the host in its 3rd instar (very rarely 4th instar), the host at that time being less than half grown. The host is killed during the process and its remains often become detached as (or soon after) the parasitoid makes a pale yellowish or buff cocoon on the plant beside it, but sometimes the two remain loosely associated (for oviposition, larval eruption and cocoon formation see https://www.youtube.com/watch?v=1mxRe-UCDxQ). The larvae of these butterflies occur in one or two generations rather early in the year and, although the parasitoid can potentially have more than one generation on suitable Pieridae (Anthocharini in part) during spring (confirmed experimentally by P. & B. Kan, unpublished; specimens in NMS), larvae of these hosts are no longer available at the time that the adult parasitoids emerge (after about six to ten days in the cocoon stage) in early to mid- summer from hosts parasitized in the late spring or early summer. The presumed subsequent hosts, which probably include one or more species with overwintering larvae inside which the *Cotesia* larva can similarly overwinter, have not been elucidated in the south of the parasitoid's range where it parasitises *Euchloe* and relatives. However, in both England and the Netherlands, where *Euchloe* species do not occur, it has been reared as a solitary parasitoid of *Aplocea efformata* (Geometridae) feeding on *Hypericum* (Hypericaceae), which has larvae that overwinter and moreover, being plurivoltine, that host species could in principle support the parasitoid throughout the year. A closely related and sometimes co-occurring species, *Aplocera plagiata*, has the same feeding habits and similar phenology and is likely also to serve as host, though unequivocal rearings from it have not been seen. But these *Hypericum*-feeding *Aplocera* species appear not to co-occur locally with its pierid hosts in at least some of the parasitoid's arid Mediterranean sites (Adela González, pers. comm.). The morphological and genetic evidence is that specimens reared from both *Euchloe* and *Aplocera* belong to a single species with an interestingly disjunct host range (cf. Shaw, 2003), but rearing experiments to confirm the host range have not been undertaken. In any case there is clearly

much to be discovered about the life history of this parasitoid in the Mediterranean region.

Cotesia parnassii Shaw sp. nov.
(Figs 3, 4; habitus Fig. 3A)

Diagnosis. This dark and moderately strongly sculptured species belongs in the first half of couplet 2 of Nixon's (1974) key, but then does not easily progress. The following combination of characters will separate it from all of the West Palaearctic *Cotesia* (as *Apanteles*) species keyed by Nixon (1974), Papp (1986, 1987, 1990) and Kotenko & Tobias (1986): (♀) antenna as long as body, with preapical segment virtually twice as long as wide; malar space long, about 1.3× as long as width of base of mandible; palpi infuscate and tegula blackish; mesonotum matt and coarsely rugose-punctate, strongest posteriorly; scutellum matt with coarse punctures strongest anteriorly; hind coxa only moderately sculptured and matt; hind femur black with its extreme base and trochantellus partly yellow/reddish below; hind tibia reddish but blackish in apical 0.4, its inner spur just reaching middle of basitarsus; fore wing weakly darkened, metacarp short, 1.8× its distance to apex of marginal cell, radius issuing from dark pterostigma only a little distal from its middle; hind wing with margin of vannal lobe with well-developed fringe of setae; 1st metasomal tergite short, as wide as long and strongly raised medially where it turns over, basal field occupying practically the whole of 2nd tergite, strongly sculptured and over twice as wide as long; 2nd tergite about 0.7× as long as third which is matt but scarcely sculptured and rather sparsely setose over most of its surface except medioanteriorly; ovipositor sheath narrow, parallel-sided and almost straight, shiny, sparsely setose along lower edge but practically glabrous above (this unusual feature is equally clear in the two available female specimens), quite strongly projecting beyond hypopygium; which is roundly angled at about 90° but not truncate apically, not projecting beyond apex of metasoma and about 0.8× as long as hind tibia.

MATERIAL EXAMINED

♀ Holotype 'SWITZERLAND: Julierpass 2570 m. Parnassius phoebus 11.8.2018, cocs 17.8.18, em. 21.8.2018 T. Kasiske. 2 Broods ⅀ 12 cocs, 2♀2♂' (in National Museums of Scotland, Edinburgh). Paratypes (1♀, 2♂) with same data as holotype (NMS).

Description

Holotype ♀. Length of body 3.1 mm, of fore wing 2.9 mm. Head in dorsal view (Fig. 3F) 1.7× as wide as long, widest across eyes, temple 0.8× as long as eye and roundly narrowing behind eye. Ocelli in a rather low triangle, the tangent to posterior pair just touching anterior one, POL 2.0×, OOL 1.8×, and distance between anterior and posterior ocellus 0.6× posterior ocellar diameter, respectively. Frons rather smooth and shiny, vertex rugulose-punctate and matt. Face (Fig. 3E) 1.4× as wide as high (excluding clypeus), coarsely but not strongly rugulose-punctate, moderately shiny, eyes not quite extending to upper margin of clypeus, not convergent at lower half of face; malar space (Fig. 4A) long, about 1.3× width of base of mandible. Antenna (Fig. 3G) 1.1× as long as fore wing, slender, its penultimate segment 1.8× as long as wide. Mesosoma 1.4× as long as high. Mesoscutum (Fig. 4D) coarsely rugose-punctate, the sculpture heaviest posteriorly where the weakly indicated notaulic courses coalesce, matt even posteriorly and on lateral lobes, evenly but not especially conspicuously setose; prescutellar suture (Fig. 4D) rather wide, with about eight fovea; scutellum (Fig. 4D) matt with distinct coarse punctures, strongest anteriorly. Mesopleuron (Fig. 4A) anteriorly matt with clear coarse punctures; precoxal area impressed, transversely finely striate, area above
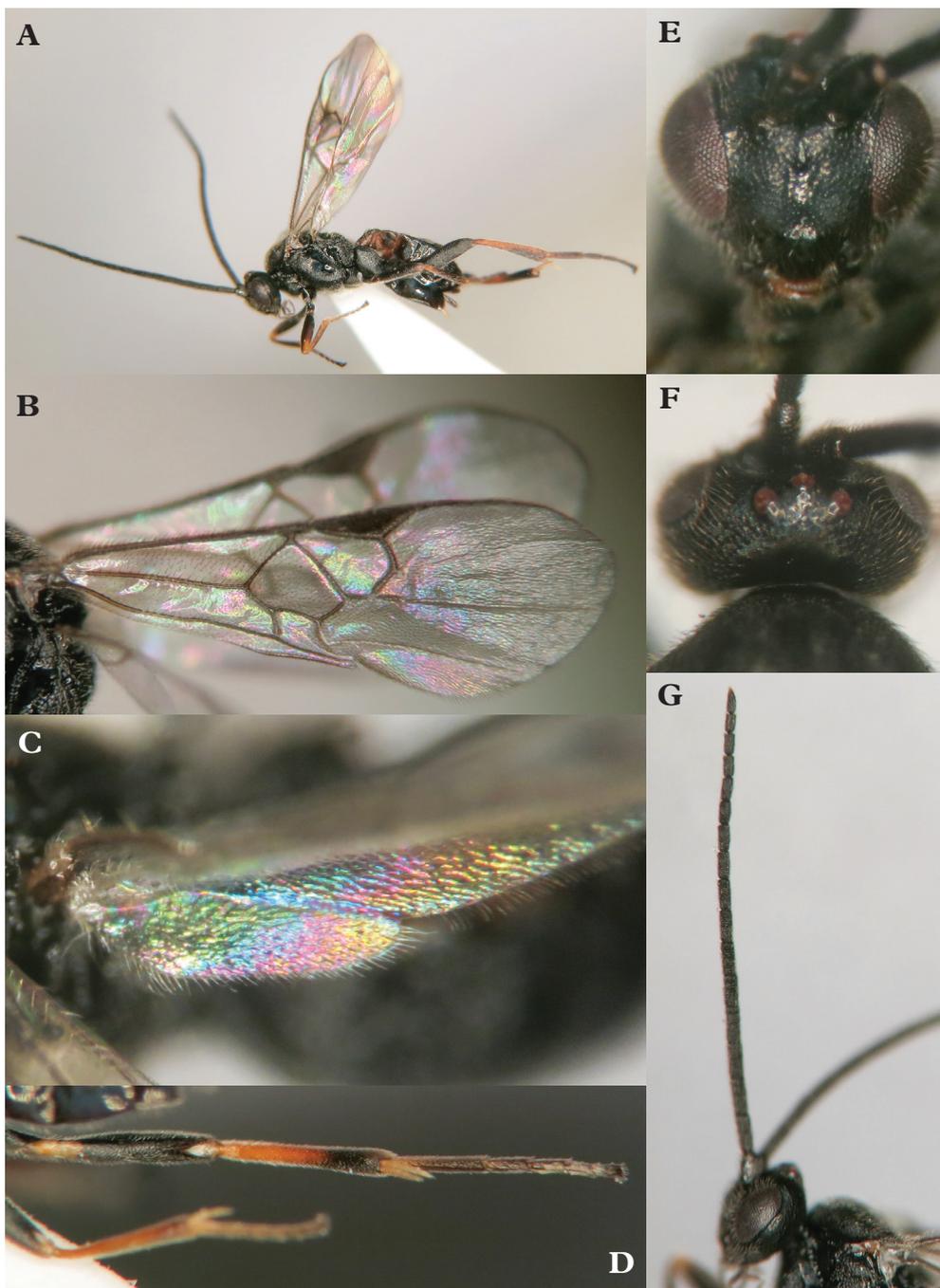
Fig. 3. *Cotesia parnassii* sp. nov. Holotype (**B**, **C**, **D**, **E**); paratype (**A**, **F**, **G**). **A**, habitus, lateral; **B**, fore wing; **C**, vannal lobe of hind wing; **D**, hind leg, ventral; **E**, face; **F**, head, dorsal; **G**, antenna.
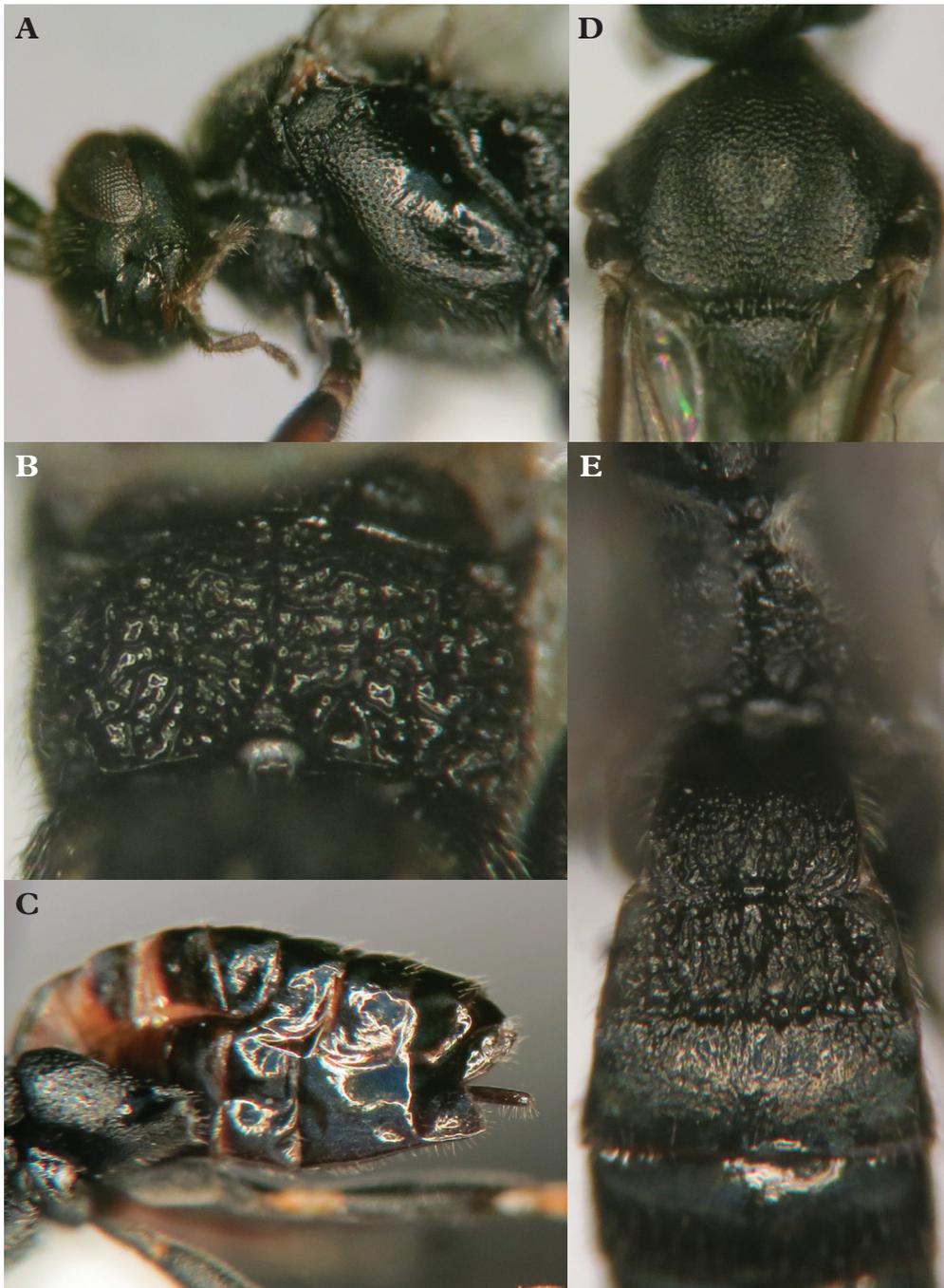
Fig. 4. *Cotesia parnassii* sp. nov. Holotype (**A**, **C**, **D**); paratype (**B**, **E**). **A**, head and mesosoma, ventrolateral; **B**, propodeum; **C**, metasoma, lateral; **D**, mesoscutum and scutellum, dorsal; **E**, anterior part of metasoma, dorsal.

Table 1. Details of the specimens appearing in Fig. 5. The specimen codes are as affixed to specimens; in three cases a specimen in NMS from the same gregarious brood as the sequenced specimen in CNC is indicated as a voucher for the same sequence (with clear labelling reflecting that). Further, the data given in Fig. 5 is sometimes in a slightly different form (e.g. MRS 0042, reflecting how it appears in BOLD, rather than MRS_JFT0042 as on the specimen in NMS).

| NMS/CNC Specimen code	Host/Country site	Identity	Sequence code in BOLD (base pairs)
MRS_JFT0343	*E. crameri*/France 1	*C. euchloevora* sp. nov.	BCNCA181-18 (618)
MRS_JFT0461	*E. tagis*/Spain 2	*C. euchloevora* sp. nov.	MRS/JFT0461 (407)
MRS_JFT0600	*E. belemia*/Spain 1	*C. euchloevora* sp. nov.	AAHYM605-16 (658)
MRS_JFT0595	*Z. eupheme*/Spain 1	*C. euchloevora* sp. nov.	AAHYM603-16 (421)
MRS_JFT0603	*Z. eupheme*/Spain 1	*C. euchloevora* sp. nov.	AAHYM606-16 (608)
MRS_JFT0064	*Ap. efformata*/England 1	*C. euchloevora* sp. nov.	ASQSR302-11 (658)
MRS_JFT0572	*Ap. efformata*/Netherlands	*C. euchloevora* sp. nov.	AAHYM583-16 (658)
MRS_JFT0200	*Am. punctidactyla*/France 2	*C. pilicornis* (Thomson)	MRS_JFT0200 (635)
MRS_JFT0203	*Am. punctidactyla*/France 2	*C. pilicornis* (Thomson)	BCNCA178-18 (652)
MRS_JFT0208	*Am. acanthadactyla*/England 2	*C. pilicornis* (Thomson)	BCNCA180-18 (621)
MRS_JFT0042	*Colias phicomone*/France	*C. ancilla* (Nixon)	ASQSR274-11 (658)
MRS_JFT0043	*Lysandra coridon*/France	*C. tenebrosa* (Wesmael)	ASQSR237-11 (658)
CAM0714	(Not reared)/Canada	*C. melanoscela* (Ratzeburg)	ASCNC099-09 (658)
MRS_JFT0014/ WAM 0432	Austria	*C. abjecta* (Marshall)	WOMIA127-11 (658)
MRS_JFT0065	(Not reared)/Wales	*C. zygaenarum* (Marshall)	ASQSR236-11 (658)
MRS_JFT0026/ WAM 0436	*Euphydryas maturna*/Poland	*C. acuminata* (Reinhard)	WOMIA131-11 (658)
MRA_JFT0006/ WAM 0401	*Vanessa cardui*/Spain	*C. vanessae* (Reinhard)	WOMIA096-11 (658)
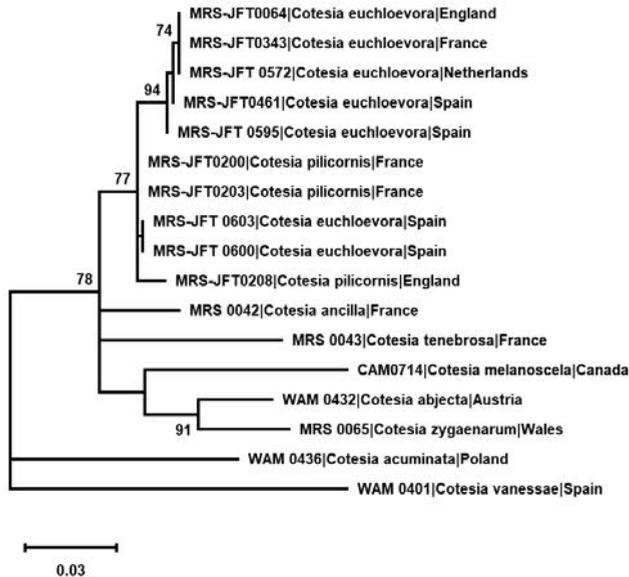
Fig. 5. Maximum likelihood tree with the highest log likelihood for 17 DNA barcode sequences of nine *Cotesia* species (including seven sequences from *C. euchloevora* and three sequences of *C. pilicornis* that compromise the molecular monophyly of the former). Tree drawn to scale, with branch lengths measured in the number of substitutions per site. Only bootstrap support of 74% or above is shown. Details of every specimen (including voucher code) and sequences used in the analysis are shown in Table 1.

that smooth and shiny, below that dull and narrowly impunctate but towards mesosternum becoming progressively rugulose. Phragma of scutellum concealed. Propodeum (Fig. 4B) very coarsely rugose but median longitudinal carina distinct. Metasoma (Fig. 4E) with 1st tergite as long as wide, strongly humped medially where it turns over preapically, where strongly rugose; 2nd tergite $2.3\times$ as wide as long, as strongly rugose as 1st or more so, basal field virtually coextensive but sublateral depressions indicating a trapezoidal area weakly evident; 2nd tergite $0.7\times$ as long as 3rd, the suture between them deep, coarsely foveolate; 3rd tergite matt, obscurely weakly rugulose in anterior third, evenly but not densely setose but glabrous medio-subanteriorly; remaining tergites shiny; hypopygium (Fig. 4C) about $0.8\times$ as long as hind tibia [the apparent lateral compression subapically is an artefact not present in the female paratype], apically descending at 90° and not truncate, not projecting beyond apex of metasoma, ovipositor sheath (Fig. 4C) narrow and parallel-sided, projecting quite strongly beyond apex of hypopygium, distally setose for a length about $0.2\times$ as long as hind basitarsus, the setae not especially concentrated apically, projecting vertically downwards, and sideways, but practically absent on upper side [the female paratype identical in this respect]. Fore wing (Fig. 3B) with pterostigma about $3.1\times$ as long as wide, emitting radius at about 0.6 its length, metacarp about $1.8\times$ as long as its distance from apex of radial cell; setae of basal cell dark and almost evenly distributed, although rather sparse. Hind wing vannal lobe (Fig. 3C) with distinct fringe of setae. Hind leg with coxa matt (Fig. 3A), weakly rugulose-punctate on outer side; femur $4.0\times$ as long as wide, matt and with indications of large shallow punctures; tibia neither much curved nor abnormally flared apically, some of the spines on outer side broad; inner spur (Fig. 3D) slightly longer than outer and reaching middle of basitarsus. Fore leg without spine on telotarsus.

Colour. Black including tegula and humeral plate, and palpi more or less. Mandible apically reddish. Metasoma anteriorly somewhat orange at sides. Fore leg with much of femur along its length and entire tibia, mid leg with tibia except apically, hind leg (Fig. 3D) with femur at extreme base (below), trochantellus and tibia in basal 0.6 (more, on dorsal side), yellow-orange (more orange-red in paratypes). All tarsi more or less infuscate. Wing membrane a little darkened, venation and setae grey to blackish.

Male. Similar to female except for sexual differences.

Molecular data (DNA barcoding). Not available at present, but it is intended that the two paratype males (labelled MRS_JFT0780 and MRS_JFT0781) will be sequenced.

Etymology. The specific epithet is a noun, from the genitive singular of *Parnassius*, the host genus.

Biology. The four available specimens resulted from two combined broods (totalling 12 cocoons) from part-grown (perhaps 4th, possibly 5th, instar) caterpillars of *Parnassius phoebus* (Fabricius) (Papilionidae), the remainder failing to emerge. Host remains and the pale yellow loosely aggregated cocoons are preserved with the paratype specimens. Apart from the data (see Material) nothing more can be said, except to remark that as far as we are aware there are no previous rearing records of a *Cotesia* species from *Parnassius*, and in fact none from Parnassiinae (at least in Europe).

A new synonymy in *Cotesia*

Through the kindness of Sergey Belokobylsij, a few years ago MRS was able to examine the holotype female of the nominal *Apanteles acutulus* Tobias, 1973 with the data '15 VI 904' ; 'W. Russland Jurburg Winogradoff-Nikitin' housed in ZIN. Apart from being a little faded through age it agrees very closely with *Cotesia cajae*

(Bouché, 1834) as interpreted by Nixon (1974, as *Apanteles*), which is a well-known parasitoid of *Arctia caja* (Linnaeus), and in MRS's opinion the nominal *Apanteles acutulus* (now *Cotesia acutula*) clearly belongs to that species (**syn. nov.**). Accordingly, MRS added a label 'Cotesia ♀ cajae (Bouché) det. M. R. Shaw, 2010' to the holotype of the nominal *Apanteles acutulus* Tobias. *Cotesia acutula* (as *Apanteles acutulus*) has been incorrectly placed in various keys (e.g. Papp, 1986, 1987; Kotenko & Tobias, 1986) because the holotype specimen is mounted in such a way as to make the hind tibial spurs very difficult to make out, but careful examination reveals that the inner one is much longer than it at first appears and, if that is properly appreciated, it would run smoothly to *cajae*. Although it has been suggested that *Cotesia perspicua* (Nees) might be the valid name for this species, Fernandez-Triana *et al.* (2020) rejected that in favour of retaining the name *C. cajae* (which, in addition, is clearly explicit in referring to its host). Incidentally, Nixon (1974) regarded this species as univoltine but in fact experiments conducted by one of us (MRS, unpublished; material deposited in NMS) show that it can have up to three generations a year on the same host generation, in a manner well-known in several *Cotesia* species.

Acknowledgements

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