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Notes on the three species of *Cotesia* Cameron, 1891 (Hymenoptera: Braconidae, Microgastrinae) parasitizing *Gonepteryx* [Leach, 1815] species (Lepidoptera: Pieridae) in Europe, with description of a new species from the Balearic Islands

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Abstract

Cotesia balearica Shaw & Colom sp. nov. is described as a solitary endemic parasitoid of *Gonepteryx cleopatra* in the Balearic Islands. A key is given to the three known *Cotesia* parasitoids of *Gonepteryx* in Europe, and biological notes are given for each species. Some new country records are given.

Key words: *Cotesia balearica*, *Cotesia gonepterygis*, *Cotesia risilis*, *Gonepteryx rhamni*, *Gonepteryx cleopatra*, rearing records, phenology, France, Spain, Sweden

Introduction

European butterflies are frequent victims of Microgastrinae, including a large number of species of *Cotesia* (Shaw, Stefanescu & Nouhuys 2009, Shaw 2009, 2022, Shaw & Fernandez-Triana 2020, Toro-Delgado *et al.* 2022), and a few are parasitized by more than one. Mostly, the *Cotesia* concerned are taxon specialists, using just one or a few closely related butterfly species, but some use other Lepidoptera at other times of year – in a few cases this is deduced from the parasitoid and host phenology without the alternate non-butterfly host having yet been discovered. Both univoltine and plurivoltine *Cotesia* species parasitize butterflies and most are gregarious, but several – including all three of the species using *Gonepteryx* treated here – are strictly solitary (Shaw, Stefanescu & Nouhuys, 2009).

Hitherto two species of *Cotesia*, *C. gonepterygis* (Marshall, 1898) and *C. risilis* (Nixon, 1974), were known as parasitoids of *Gonepteryx* in Europe, both originally described as parasitoids of *G. rhamni* (Linnaeus, 1758) in England. The two *Gonepteryx* species that occur in western Europe are essentially univoltine and have extremely similar life cycles; overwintering as adult butterflies, ovipositing on *Rhamnus* species in spring, with indistinguishable larvae developing fairly rapidly and the resulting adult butterflies entering hibernation before autumn. The *Cotesia* species oviposit into early instar larvae, and typically kill the host in its third instar. While *C. gonepterygis* is strictly univoltine and clearly synchronised with its only known and univoltine host, suggesting that it needs no other, *C. risilis* appears to be plurivoltine and to require other hosts to complete its annual life

cycle (see later). Both parasitoids are known in several other European countries (Fernandez-Triana *et al.* 2020), often clearly associated with *G. rhamni* when only that species of *Gonepteryx* is present but, owing to the close similarity of larvae of *Gonepteryx* species in phenology and larval foodplant as well as in appearance, when more than one species is present the exact host is generally unclear. As far as we are aware no *Cotesia* has yet been recorded from the eastern European *G. farinosa* (Zeller, 1847).

Fieldwork by one of us (PC) in N. E. Spain, where both *Gonepteryx cleopatra* (Linnaeus, 1767) and *G. rhamni* occur, and the Balearic Islands where only *G. cleopatra* is found, has revealed a third *Cotesia* species, described as a new species below, parasitizing *G. cleopatra* in both Menorca and Mallorca, but not in the Spanish mainland. Neither *C. gonopterygis* nor *C. risilis* appears to be present in the Balearic Islands (Colom *et al.* in prep.).

Materials and methods

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

Depositories

National Museums of Scotland, Edinburgh (NMS)

The Natural History Museum, London (NHMUK)

Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa (CNC)

Mediterranean Institute for Advanced Studies (IMEDEA, UIB-CSIC), Esporles, Mallorca, Spain (MIAS)

Natural Sciences Museum of Granollers, Granollers, Spain (NSMG)

DNA Barcoding

Standard protocols outlined in Shaw (2022) were applied, at CNC and the Canadian Centre for DNA Barcoding (Guelph, Ontario). CO1 sequences are deposited in the Barcode of Life Data Systems, <http://www.boldsystems.org/> (BOLD), with the sample references indicated below.

Cotesia balearica Shaw & Colom, **sp. nov.**

(Figs 3, 5–16)

Diagnosis. In Nixon's (1974) key the new species will run rather smoothly to couplet 53 where it differs from *C. numen* (Nixon, 1974) in the shape of the pterostigma and the length of the propodeum, and from *C. gonopterygis* most obviously in the characters given in the key below. In Papp's (1987) key it runs to couplet 138/161 then, if the metacarp is judged to be not longer than the pterostigma, to *C. gonopterygis* or, if judged longer, then best to *C. juniperatae* (Bouché, 1843). [The relevant ratio depends on where the distal end of the pterostigma is judged to become the metacarp: in both *gonopterygis* and the new species the ratios are here reckoned about the same, with the metacarp marginally

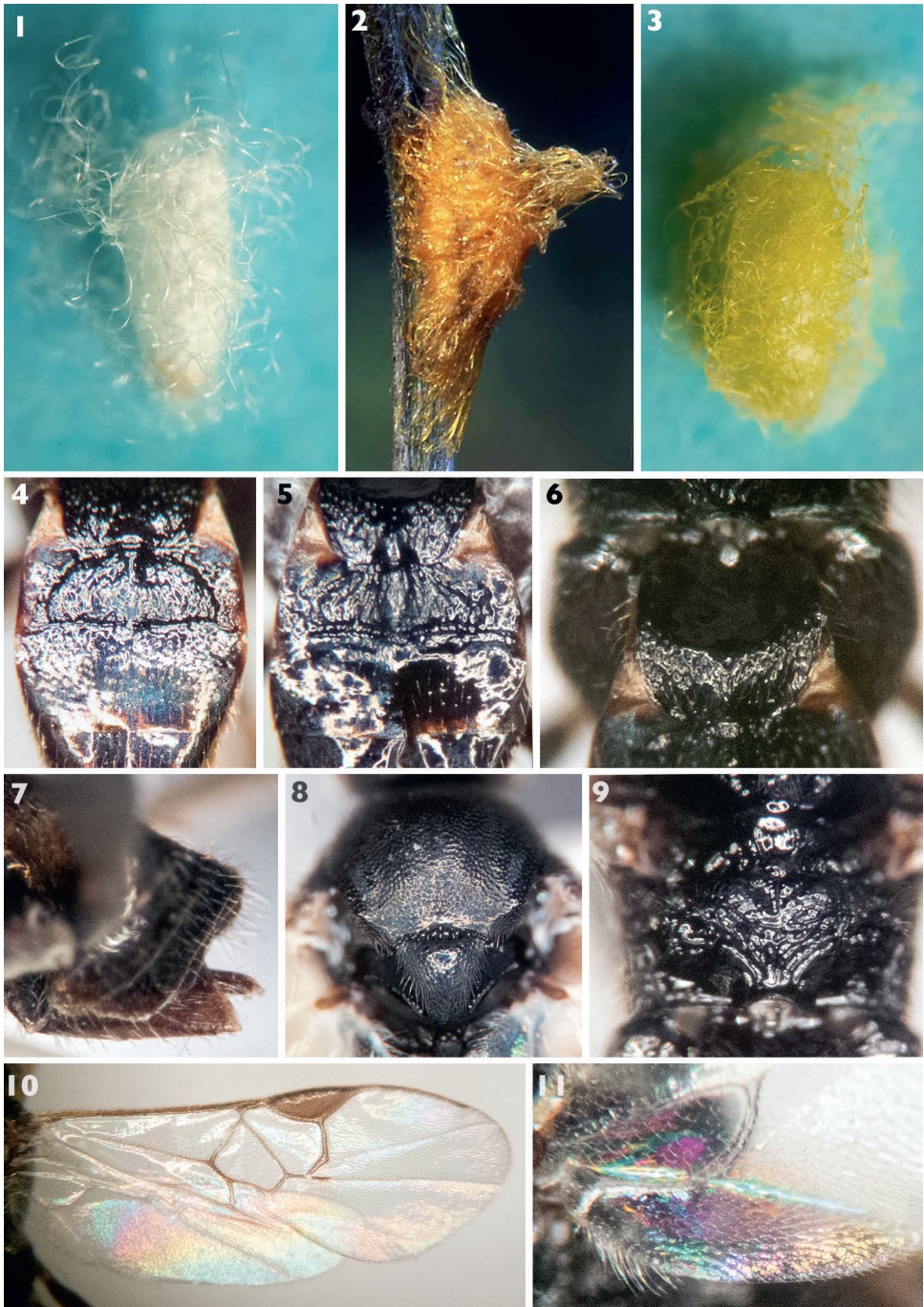
the longer]. Although *C. juniperatae* is a rather ill-understood (*cf.* Fernandez-Triana *et al.* 2020) and, according to Nixon's (1974) characterisation, variable species, it usually has the preapical antennal segment shorter, the first metasomal tergite less roundly narrowed, the second tergite less transverse, the hypopygium more truncate and the propodeum with a stronger medial keel as well as darker legs than the new species. However, it is possible that when the summer/autumn generation of the new species is found, possibly with overwintering cocoons, it might be misidentified as *C. juniperatae*. Identity with that can, however, additionally be ruled out because nothing resembling the new species has been found in the European mainland or indeed Britain (despite many rearings of *G. rhamni* larvae over the years) in areas harbouring *C. juniperatae* (see Fernandez-Triana *et al.* 2020 for country distributions).

The cocoon of the new species, at least in early summer, with its open net of surrounding strands, is similar to that of *C. risilis* but strongly lemon yellow in colour rather than pure white, and also tends to be less extreme in the extent of its outer covering. While the cocoon is extremely constant in its appearance, the adult is a rather variable species.

Description

Holotype ♀, length 2.7 mm, of fore wing 3.1 mm. Head in dorsal view 2.0× wider than long; temple 0.9× as long as eye, roundly narrowing; OOL 2.6× and POL 2.1× diameter of posterior ocellus; ocelli in low triangle, tangent to posterior ocelli just cutting anterior ocellus; stemmaticum and frons almost smooth, shining; vertex and temple very weakly rugulose; face 1.5× wider than high, weakly rugulose, moderately shiny; clypeus smoother and shining; eyes about parallel, extending to level of tentorial pits; malar space as long as basal width of mandible; antenna as long as fore wing, preapical segment twice as long as wide. Mesoscutum weakly rugose, with punctate element poorly developed and scutellum similar, more shining; prescutellar sulcus deep, crenulate; phragma of scutellum moderately exposed; mesopleuron weakly sculptured, anterior margin rugulose with only indistinct small punctation, precoxal area with rugae indicated; propodeum (variable) with most rugae more or less directed upward and outward from near articulation with metasoma and very often only very weak anterior development of a median carina, posterior lateral areas weakly sculptured and shining (in a few cases propodeum almost unsculptured except for at most a weak median carina). Fore wing with setae fairly evenly distributed in basal and subbasal cells; pterostigma unexceptional in shape, about 2.4× as long as deep, usually emitting r only slightly distal from middle (but rarely up to two thirds); metacarp about 3.5× as long as its distance from apex of marginal cell. Vannal lobe of hind wing with virtually no setal fringe near widest part. Hind leg with coxa weakly sculptured both dorsally and laterally, moderately shining, femur about 3 times longer than wide, tibia slightly curved but evenly widening towards apex and about 5× as long as apical width, tibial spurs almost equal and not reaching middle of basitarsus. Fore leg without spine on apical tarsal segment. Metasoma with T1 as long as its greatest width, smooth and shining in deeply excavate anterior part, strongly humped at narrow apical turned-over part which is irregularly longitudinally rugose (though sometimes weakly) with some punctures; T2 about 3.3× wider than long with more or less rugulose sculpture (often very weak) and a triangular basal field not reaching full width of tergite and usually with lateral sulci poorly indicated but sometimes stark; T3 about 1.5× as long as T2 and just under half as long as wide anteriorly, rather evenly setose and with little or no sculpture in anterior quarter; hypopygium 0.5–0.6 as long as hind tibia, descending at about 60° or much less (prone to artefact), often roundly but not truncate apically; ovipositor sheath short, often only weakly protruding in death.

Black; palpi apically whitish, labrum and mandible apically brown or sometimes paler. All trochanters and especially trochantelli partly yellowish brown and tarsi darkening apically; fore leg yellow except base of femur washed brown; mid leg with femur brownish on basal two thirds, otherwise largely yellow; hind femur blackish or dark brown (sometimes weakly orange



Figs 1–11. *Cotesia* species. 1–3 cocoons. 1, *C. risilis*; 2, *C. gonopterygis*; 3, *C. balearica* sp. nov. 4, 5 metasomal tergites 2+3, female. 4, *C. gonopterygis*; 5, *C. balearica*. 6–11 *C. balearica*, female. 6, apex of first metasomal tergite; 7, hypopygium and ovipositor sheath; 8, mesoscutum and scutellum; 9, propodeum; 10, wings; 11, vannal lobe of hind wing.



Figs 12–16. *Cotesia balearica* sp. nov., female. 12, habitus; 13, head, dorsal; 14, face; 15, hind leg; 16, antenna.

centrally) and tibia yellowish with apical third becoming brown especially in lower part, spurs yellow or ivory, tarsi brownish except base of first segment yellow. Colour of yellowish parts of legs varies from orange to almost ivory. Wings with pterostigma and most of surrounding venation brown; costa becoming yellow proximally.

Male. Like female except for sexual characters.

MATERIAL EXAMINED

Holotype ♀ 'MALLORCA: Calvià, Es Burotell. *Gonepteryx cleopatra* 2.4.2021em 23.4.21 P. Colom' (in NMS). Paratypes (15♀, 15♂, in NMS unless stated otherwise). 9♀, 7♂ with data as holotype but various collection and emergence dates in spring 2020 and 2021 (1♀, 1♂ in NHMUK; 1♀, 1♂ in CNC; 1♀ in MIAS; 1♂ in NSMG); 6♀, 1♂ Mallorca, Bunyola, Raixa ex *G. cleopatra* with various collection and emergence dates in spring 2020, P. Colom (1♀ in NSMG); 4♂ Mallorca, Establiments ex *G. cleopatra* with various collection and emergence dates in spring 2020, P. Colom (1♂ in MIAS); 1♂ Mallorca, Escorca, Binifaldó ex *G. cleopatra* coll. 24.iv.2021, em. 2.v.2021, P. Colom; 1♂ Mallorca, Alcudia, Ses Puntes ex *G. cleopatra* coll. 5. iv.2021 em. 27.iv.2021, P. Colom; 1♂ Mallorca, Esporles, ex *G. cleopatra* coll. 11.iv.2019, em. iv/v.2019, P. Colom.

Key to the *Cotesia* species known to parasitize *Gonepteryx* species in Europe

The three species involved share several features: head rather transverse, with ocelli in a low triangle; moderately long antenna with preapical segment at least 1.5× its width; mesoscutum with rather weak sculpture and almost obsolescent punctation, uniformly extending to its posterior margin; setae of median cell of fore wing fairly evenly distributed; vannal lobe of fore wing virtually lacking setal fringe beyond its widest part; apical segment of front tarsus lacking specialised spine; hind femur black(ish); phragma of scutellum slightly exposed; third tergite of metasoma with setae distributed over most of its surface. Most of the European species sharing these characteristics are solitary, as is the case with all three of the species keyed below (the key is only for *Cotesia* parasitoids of *Gonepteryx* and, for reared specimens, the great difference in the cocoons is particularly striking).

- 1 First metasomal tergite slightly widened towards apex; basal field of second tergite more or less rectangular and occupying almost entire tergite; hind tibial spurs unequal, the inner one reaching just beyond middle of hind basitarsus. (Cocoon white, enclosed in an open net, Fig. 1) *risilis* (Nixon)
- First tergite roundly narrowing towards apex; basal field more triangular with more or less distinct lateral sulci; hind spurs equal, not extending beyond middle of basitarsus. (Cocoon yellow or golden orange, Figs 2, 3) 2
- 2 Second tergite less transverse and with basal field clearly defined by lateral sulci (Fig. 4), third tergite longer (at least half as long as wide anteriorly); wing membrane slightly tinged brownish. (Cocoon golden orange, compact, with distinct mid-dorsal tuft, Fig. 2)
. *gonepterygis* (Marshall)
- Second tergite more transverse and with basal field usually rather ill-defined (Fig. 5), third tergite shorter (less than half as long as wide anteriorly); wing membrane colourless. (Cocoon lemon yellow, enclosed in an open net, Fig. 3) *balearica* Shaw & Colom, **sp. nov.**

Biological notes on the species

(* denotes a new country record, according to Fernandez-Triana *et al.* 2020)

***Cotesia risilis*.** This is a regular solitary parasitoid of *G. rhamni* in early summer, with rapid emergence of the adult, over much of the range of that host. In NMS there are reared specimens from England, France*, Italy, Spain* and Sweden*. In most cases the host was clearly *G. rhamni* but one specimen from N. E. Spain was judged by its rearer (C. Stefanescu) to be from *G. cleopatra*, and was recorded as such by Shaw, Stefanescu and Nouhuys (2009). However, partly in view of the lack of corroboration by further rearing effort in the same general area of Spain by PC where *G. cleopatra* was the dominant species (Colom *et al.* in prep.), we now regard this association as probably incorrect, and certainly in need of confirmation. Because the adult has invariably hatched quite soon after the cocoon was made (usually on a leaf of the foodplant) in all rearings from *Gonepteryx*, and because it is inconceivable that an adult *Cotesia* could survive from early summer until the following spring, it seems clear that at least one further generation occurs, either in a host in which the winter can be passed or the *Cotesia* then passing the winter in a cocooned stage – but the host (or hosts) involved have not been discovered. A further early summer host is *Satyrrium w-album* (Knoch, 1782) (Lycaenidae), evidenced by a single male (barcoded, specimen in NMS) reared by P. & B. Kan in S. France. *Cotesia risilis* (as *Apanteles*) can be identified through Nixon's (1974) key.

***Cotesia gonepterygis*.** In contrast to the other two species dealt with here, this is a univoltine parasitoid completely phenologically attuned to its *Gonepteryx* host, the winter being passed as a prepupa in a tough cocoon specialised for overwintering that is spun on a twig of the foodplant. In N. E. Spain* it is a regular solitary parasitoid of *Gonepteryx* in areas where *G.*

rhamni and *G. cleopatra* co-occur, but DNA analysis of host remains [sequences deposited in BOLD are publicly available at <https://doi.org/10.5883/DS-GONCOLOM>] has not yet shown that *G. cleopatra* serves as host, all 12 samples so far analysed proving to be *G. rhamni* although that was the less common host locally: ten were from a site where the ratio of *rhamni* to *cleopatra* larvae was 0.6 (n=83), and the other two from a site where the ratio was only 0.04 and n=130. Thus the inclusion of *G. cleopatra* as host by Shaw, Stefanescu & Nouhuys (2009) should be regarded as almost certainly an error. *Cotesia gonopterygis* (as *Apanteles*) can be identified through Nixon's (1974) key, though his figure 74 is a little misleading (compare with Fig. 4).

***Cotesia balearica* sp. nov.** Both in Menorca and Mallorca, PC has reared this as a regular and moderately abundant solitary parasitoid of *G. cleopatra*, the only *Gonepteryx* species that occurs in the Balearic Islands.

The parasitoid was found in all ten of the investigated sites, in which it occurred in all years of the study (Colom *et al.* in prep.). Host larvae were generally collected in the first three instars in early April, with parasitoid eruption from L2 or L3. Depending on conditions, adults emerged from the cocoon after about 7 to 12 days, later in April or in some cases during the earliest days of May. The cocoon is generally formed exposed on a leaf of the foodplant, facilitating rapid emergence. Thus the phenology of *C. balearica*, and hence the need for a further host, matches that of *C. risilis*, and again there is no knowledge of how the annual life cycle is completed. It appears to be a Balearic Islands endemic.

Barcode distances of the three species parasitizing *Gonepteryx*

CO1 sequences have been obtained for all three species, involving reared material housed in NMS. The Sample ID (voucher codes) referenced in BOLD are as follows: *C. risilis* MS 095 (ex *Gonepteryx*) and MRS-JFT0604 (ex *Satyrium*); *C. balearica* MRS-JFT 0899, MRS-JFT 0900, MRS-JFT 0901; *C. gonopterygis* MS 079, MRS-JFT 0586 (further samples not yet processed, based on prepupae extracted from the characteristic cocoon, will be referenced MRS_JFT1019, MRS_JFT1020, MRS_JFT1021 and MRS_JFT1022 if successful).

Based on the above, the barcode distance between *C. risilis* and *C. gonopterygis* is only around 1.2%, with this pair about 4.5% distant from *C. balearica*. It is perhaps surprising that *C. gonopterygis* and *C. risilis* appear to be closely related, while *C. balearica* is more divergent.

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