

## RESEARCH PAPER

## New data on the early stages and behaviour of the endangered species *Callophrys mystaphia* (Lepidoptera: Lycaenidae) and its first larval parasitoid, *Cotesia* sp. (Hymenoptera: Braconidae)

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**Abstract.** The early stages of a lycaenid butterfly, Miller's Green Hairstreak *Callophrys mystaphia* Miller, 1913, are photographed and presented for the first time. *Callophrys mystaphia* is known to be endangered and is regarded to be endemic to Turkey based on recent studies. The species is considered to be local and isolated, with the likelihood of being one of the rarest butterfly species in Turkey. The distribution of this species and its food plant, *Rheum ribes* L. (Polygonaceae), in Turkey are investigated and the possible range of this butterfly species is highlighted. New data on the early stages, phenology, vertical distribution, and behaviour of adult and larva of *C. mystaphia* are presented. The larva was found to have been attacked by a newly discovered hymenopteran parasitoid species of the genus *Cotesia* Cameron, 1981 (Braconidae); the cocoons and the adult parasitoid are illustrated.

**Key words.** Lepidoptera, Lycaenidae, Hymenoptera, Braconidae, biodiversity, biology, endemic, host plant, larval parasitoid, larval stages, Turkey, Palaearctic Region

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### Introduction

The genus *Callophrys* Billberg, 1820 is characterized by green colour and the presence (or absence) and arrangement of white spotting on the ventral surfaces of the wings. Within the Palaearctic *Callophrys*, seven partially heterogeneous groups of species could be identified according to a phylogeny based on the mitochondrial COI gene, with *C. mystaphia* Miller, [1913], being placed in the *C. rubi*-group (TEN HAGEN & MILLER 2010). According to KOÇAK & KEMAL (2018), the genus is represented by six species in Turkey: *Callophrys mystaphia*, *C. chalybeitincta* Sovinsky, 1905, *C. danchenkoi* Zhdanko, 1998, *C. herculeana* Pfeiffer, 1927, *C. paulae* Pfeiffer, 1932, and *C. rubi* (Linnaeus, 1758). Miller's Green Hairstreak, *Callophrys mystaphia*, was described from the Aras River Valley, Kars, Kağızman, Eastern Turkey, near the Armenian border. WAGENER & VAN OORSCHOT (1998) stated that the locus typicus, 'Mystaph', is in the same place as 'Mesta' near the village of 'Novoja Nicolaewka', which is identical to the village known today as Akçay (right bank of the Arax, on the border between the Kars and Erivan Governments at an altitude of around 1800 m). For a long period, only the

type specimens of *C. mystaphia* were known. WAGENER & VAN OORSCHOT (1998) viewed *C. mystaphia* and *C. paulae* as being conspecific, and incorrectly classified both species as *C. mystaphia*. However, investigations conducted since 1995, including a revision by TEN HAGEN & MILLER (2010) analysing the genus *Callophrys*, have supported that *C. mystaphia* (based on samples only from SW Iran) should be classified as a separate species based on morphology, ecology (especially plant preference), and mtDNA COI barcodes. The species was originally thought to be distributed in Turkey, Iran, and Afghanistan (HESSELBARTH et al. 1995). It was later realized that the populations from south-western Iran were erroneously identified as *C. mystaphia* by TEN HAGEN (2006) due to the similarity of the morphological characters. A comparison with the type specimens of *C. mystaphia* convinced KRUPITSKY & KOLESNICHENKO (2013) that specimens from south-west Iran represent a different species described as new, *C. mystaphioides* Krupitsky & Kolesnichenko, 2013. However, TSHIKOLOVETS et al. (2014) refer to the presence of *C. mystaphia mystaphia* (probably in north-west) and *C. mystaphia mystaphioides* (south-west) in Iran. Furthermore, the identification of



specimens from Afghanistan (Paghman Mts.) mentioned in HEYDEMANN (1954) as *C. mystaphia*, was determined to conform perfectly to *C. afghana* Krupitsky, Pljushtch & Pak, 2012 (KRUPITSKY et al. 2012). Thus, the distribution area of *C. mystaphia* became limited only to a small area, the east and southeast of Turkey.

*Callophrys mystaphia* and *C. mystaphioides* are thought to be closely related to the *C. suaveola* species group by KRUPITSKY & KOLESNICHENKO (2013). In contrast, TEN HAGEN & MILLER (2010, misidentified as *C. mystaphia*) discovered that *C. mystaphioides* and *C. rubi* have quite similar DNA barcodes despite significant morphological and genitalic differences between these two butterfly species. However, phylogenetic reconstructions of *Callophrys* were based solely on the COI genes, which are not always sufficient (STRADOMSKY & VODOLAZHISKY 2011).

Little is known about the biology of *C. mystaphia*; the only such study was undertaken by KEMAL (2009), employing video to investigate the developmental stages. Furthermore, there is no previously known record of any parasitoid species attacking the larval stages of *C. mystaphia*; here, details are provided of a braconid species of the genus *Cotesia* Cameron, 1891 newly discovered as a parasitoid of Miller's Green Hairstreak.

### Material and methods

**Material examined.** Three fully grown larvae of *C. mystaphia* were collected from *Rheum ribes* growing in a field alongside the Maden road, Şirvan district, Siirt Prov., southeast Turkey on 1 June 2020, at 1400 m, 38°04'30"N 42°07'11"E. Larvae were also observed in the field on 18 May 2021. Alongside these, some adult specimens collected by SEVEN (2010) were examined: 3 ♂♂ 2 ♀♀, Maden road (the same location as above), 1380–1480 m a.s.l., 26.iv.2009; 14 ♂♂ 5 ♀♀, 1.v.2009, 1 ♂, Hesko (38°08'07"N 42°01'15"E), 975 m a.s.l., 3.v.2009; and 1 ♂, Maden junction (38°03'51"N 42°04'02"E), 950 m a.s.l., 25.iv.2009, all leg. E. Seven.

The larvae, together with the food plant, were transported to the laboratory in a transparent box. Larval growth was monitored daily and throughout that process, a Fuji-film Finepix HS30EXR camera was used to photograph the various stages of development; the images were later arranged in Adobe Photoshop CS6. In addition, some adult specimens collected by SEVEN (2010) from the same location were also investigated. The material of adults

and larvae is deposited in the Entomology Laboratory of Batman University (BTU), Batman, Turkey.

**Terminology** follows MILLER (1913), KOÇAK (1977), HESSELBARTH et al. (1995), WAGENER & OORSCHOT (1998), TEN HAGEN (2006), TEN HAGEN & MILLER (2010), TSHIKOLOVETS (2011, 2014), KRUPITSKY & KOLESNICHENKO (2013), and KOÇAK & KEMAL (2018).

### Results and discussion

**Diagnosis. Adult** (Fig. 1). *Callophrys mystaphia* is the smallest member of the genus *Callophrys* in Turkey. Special features distinguish it from other *Callophrys* species, namely those of exceedingly small size, sharply rounded outer margins of all wings. *Callophrys mystaphia* is recognized from all the congeners by the complete absence of the lobe of the hind wings, the black upper surface of the wings, the perpendicular curved white submarginal line and the orthogonally curved white submarginal line (TEN HAGEN 2006, KEMAL 2009, TEN HAGEN & MILLER 2010). The wingspan is 17–18 mm. Antennae are black with white rings at the base of the segments. Greenish-white scales are surrounded by brown eyes with small, pale brown hairs. The brown androconial spot is small and rounded. The ground colour of the underside of the forewing is emerald green, and the basal area has a few greenish hairs. White postdisical spots are mostly distinct and rounded.

**Adult variation.** The white row of dots on the underside of the wings of *C. mystaphia* shows significant variation in Turkey (HESSELBARTH et al. 1995, KEMAL 2009, SEVEN 2010, TSHIKOLOVETS 2011, TRAKEL 2022). It is quite prominent in certain individuals while being less so in others. Due to these intraspecific variation, the identification of some species of this genus is difficult and confusing. External morphological and genital characters have been used to classify the populations as separate taxa in Iran (KRUPITSKY & KOLESNICHENKO 2013, TSHIKOLOVETS et al. 2014).

**Larva** (Figs 2, 3). *Callophrys mystaphia* larvae resemble woodlouse, like the majority of other members of their family. Adult larvae have a large, plump body that becomes flattened at the ends and sharply indented between the segments. The larvae have completely smooth green colour; however, they start to turn brown as they approach pupation. It has a head and 10 flattened segments. The head is round in dorsal view and slightly angular in lateral view. The segments have a prominent dorsal hump, concave sides, and a dilated lateral ridge. The head partially retracts

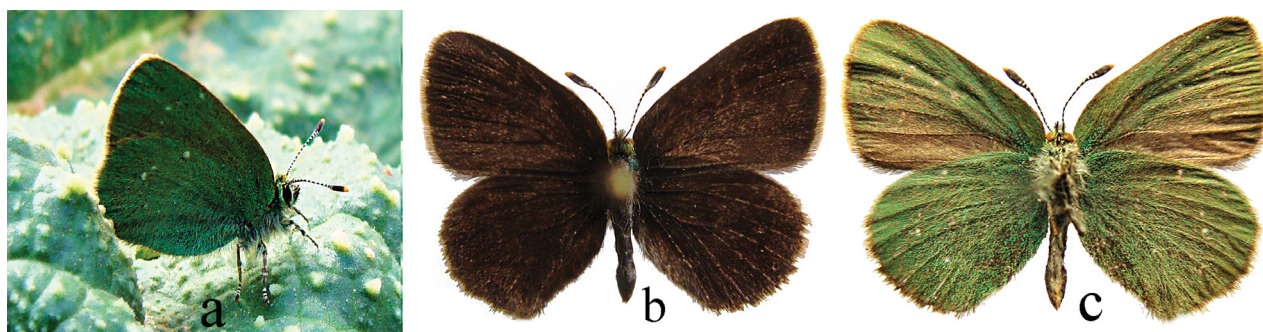


Fig. 1. Habitus of *Callophrys mystaphia* Miller, 1913 (a – 29.iv.2020 during a field study, b–c – stretched specimen).



into the first segment when in rest and feeding, and is the same shade of green as the body. There is no longitudinal line medially on dorsum. The legs are pale green. Larvae are bearing setae, the entire dorsal surface of the head, thorax, and abdomen is heavily covered with whitish, finely serrated spines of various length. There are no spines on the limbs. Raised reticulations cover the whole surface of body, and numerous tiny lenticles can be seen along the spiracular region. Spiracles are lighter in colour, they are whitish and distinct.

Except for *Callophrys rubi*, information on the larval development of the majority of species in this genus is still unknown or very limited. According to EELES (2022), the ground colour of *C. rubi* is amber-brown, checkered and speckled with black which is most conspicuous as transverse markings on the abdomen. The colour of segments is a brilliant pure green, with a series of sub-dorsal oblique citrine-yellow markings commencing on the third segment, and a lateral line of the same colour. *Callophrys rubi* is rather densely covered with brown spinous hairs of various lengths (EELES 2022). The larva of *C. mystaphia* is similar to *C. rubi*, but *C. mystaphia* is completely smooth green and has no transverse markings on the abdomen. Also, it has whitish spinous hairs.

**Pupa** (Fig. 4). In the pupal stage of *C. mystaphia*, the abdomen is rounded and smooth brown and rather fat compared with the head and thorax. The ventral part of abdomen is lighter brown. Pupae have a characteristic brown, sparse setation, except for the ventral part of the pupa. The general pupal morphology of *C. mystaphia* is similar to that of the *Callophrys* genus in general: some species have distinctive darker spots (such as *C. rubi*), especially on abdomen, but *C. mystaphia* is almost unicolorous brown. **Biology. Larva.** Three larvae of *Callophrys mystaphia* were found near Maden road at 1400 m a.s.l., on 1 June 2020 on the host plant *Rheum ribes*. Two of them pupated on 13 and 15 June 2020, respectively. One adult emerged on 23 April 2021. The second one did not emerge from the pupa which probably dried out due to the high temperature in summer months. The third larva was found parasitised (see below). Further seven larvae were found during investigations in the area of the host plant on 18 May 2021. It seems that larvae can be found from late May to mid-June in the study area.

The associations of lycaenid larvae and pupae with ants, termed myrmecophily, are mediated by epidermal glands whose secretions modify the ants' behaviour. The most widespread type of lycaenid-ant interactions involves

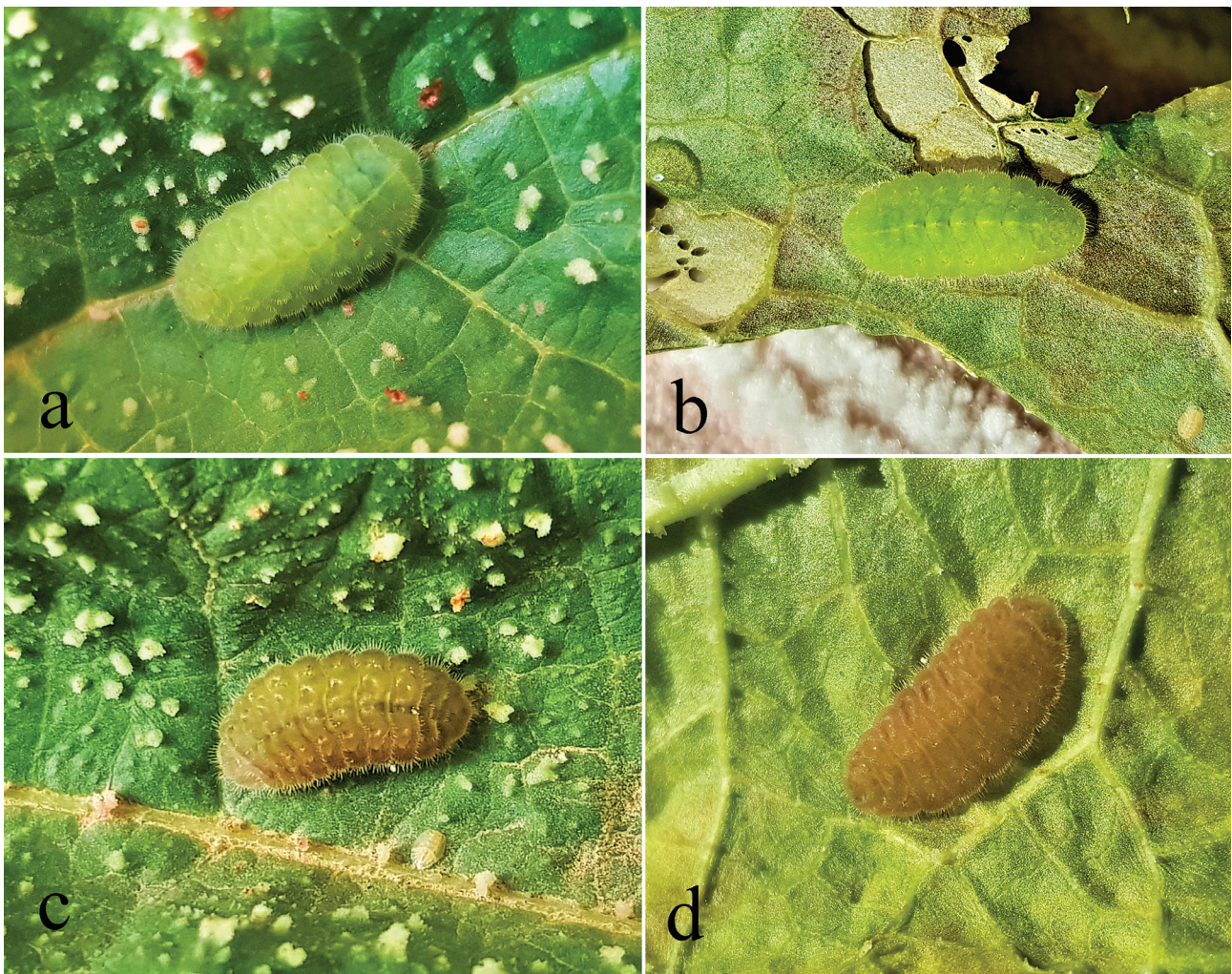


Fig. 2. Feeding larvae of *Callophrys mystaphia* Miller, 1913 on *Rheum ribes* host plant. (a–b – fully grown larvae, 20.vi.2020; c–d – final instar larvae approaching pupation, 4.vi.2020).



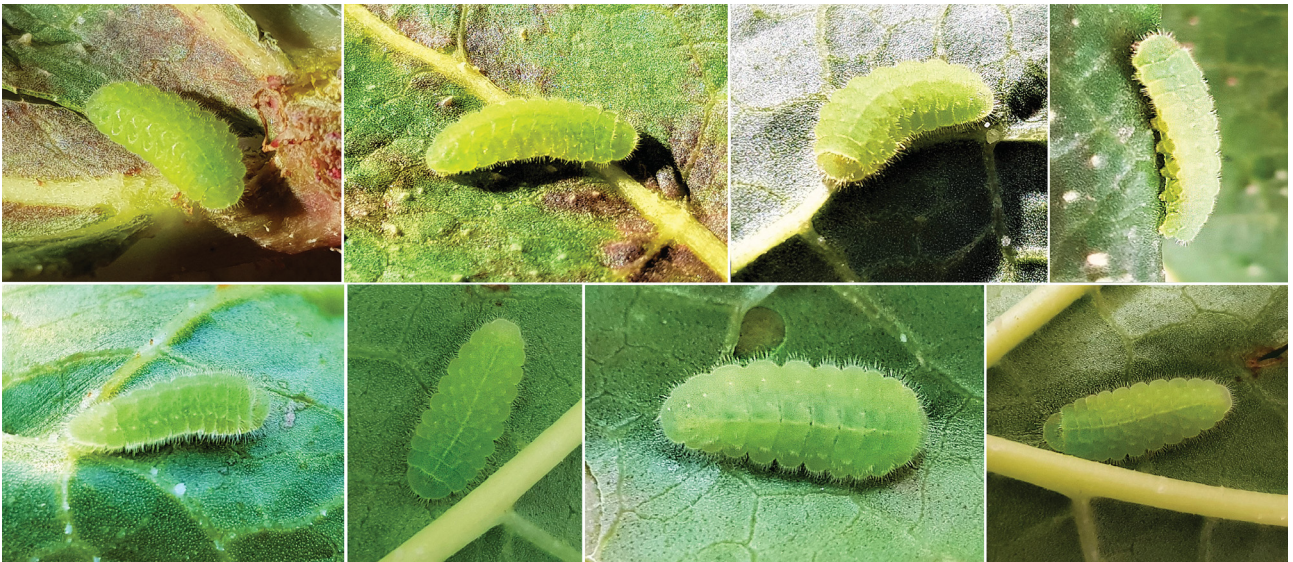


Fig. 3. Larvae of *Callophrys mystaphia* Miller, 1913 on host plant from various perspectives.

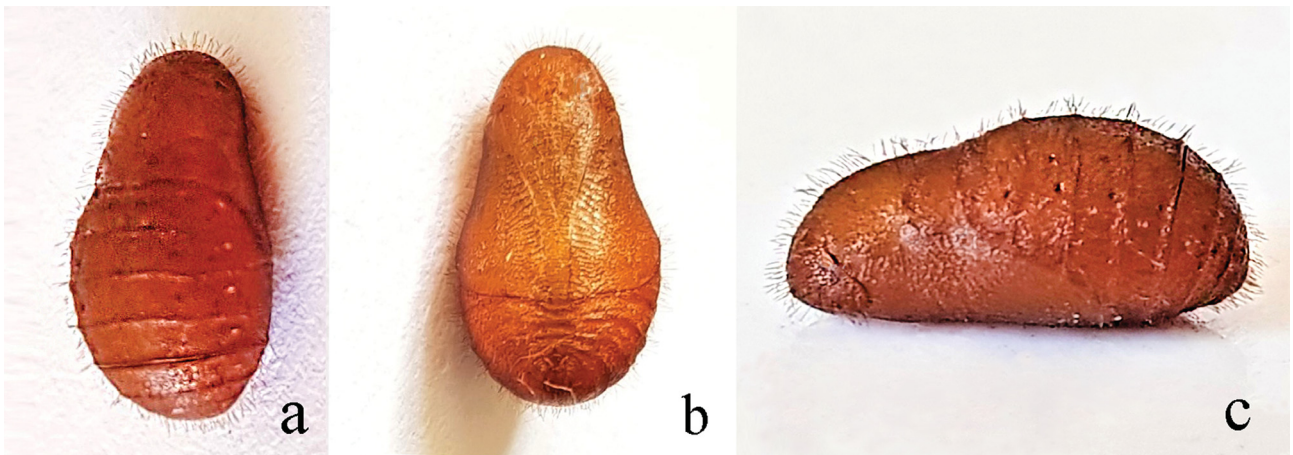


Fig. 4. Pupae of *Callophrys mystaphia* Miller, 1913 on 13.vi.2020 (a – dorsal, b – ventral, c – lateral view).



Fig. 5. Larvae of *Callophrys mystaphia* Miller, 1913 forming 'feeding windows' on the host plant.



phytophagous caterpillars while they are feeding on their host plants. These caterpillars are visited by ants to harvest their secretions. Life history data are available for only a fraction of Turkish Lycaenidae species. In many cases, these data have not been collected in Turkey but in Central Europe or the Western Mediterranean. Only a few records of myrmecophily have been documented in Turkey, and the type of ant interaction involving *C. mystaphia* remains unknown (FIEDLER 1995). No such interaction has been observed on the larvae in the study area, either. However, myrmecoxenous caterpillars are not associated with ants, and the caterpillars of Lycaenini are generally not tended by ants as in *C. rubi* (FIEDLER 1990, 1995). Perhaps *C. mystaphia* larva is also myrmecoxenous.

The larvae of *C. mystaphia* showed no tendency to cannibalism in their natural environment or laboratory conditions as in *C. rubi* (EELES 2022).

**Pupa.** The pupa is not fixed, it has a thick, rounded, and dumpy shape. The pupal stage lasted 10 months and 17 days in laboratory conditions, which is from about mid-June to the middle of the following March. They pupated on the ground in captivity and were concealed on leaves of the broom they consumed as food. They surely pupate on the ground among moss or other suitable cover in nature. In addition, it is thought that pupae maintain their moisture balance deep in the soil to protect themselves from the dry and hot summer months in the study region.

**Phenology.** *Callophrys mystaphia* is univoltine, flying from late April to mid-June (MILLER 1913, HESSELBARTH et al. 1995, KEMAL 2009, SEVEN 2010, TSHIKOLOVETS 2011). In the study area, the population was high in numbers in local sites where *R. ribes* is abundant at the beginning of May. Towards the beginning of June, the number of flying adults decreases as the weather gets warmer and the plants start to wither. *Callophrys mystaphia* was not observed in the study area later than mid-June. The species flies for 15–20 days on average. The temperature is about 19°C at the end of April when the species begins to fly, increasing to around 32°C in mid-June (TSMS 2021).

At the Maden road habitat, *C. mystaphia* was co-occurring with the following identified species of butterflies: *Anthocharis cardamines* (Linnaeus, 1758), *A. gruneri* Herrich-Schäffer, [1851], *Callophrys suaveola* (Staudinger, 1881), *Colias crocea* (Geoffroy, 1785), *Issoria lathonia* (Linnaeus, 1758) (in high numbers), *Pieris persis* Verity, 1922, *Polyommatus agestis* ([Denis & Schiffermüller], 1775) (in high numbers), *P. icarus* (Rottentburg, 1775) (in high numbers), *Pontia chloridice* (Hübner, [1813]), *Vanessa cardui* (Linnaeus, 1758), and *Zerynthia deyrollei* (Oberthür, 1869). In addition, the following species were flying together with *C. mystaphia* in the habitat of Ahlat district of Bitlis (O. Başbay, pers. comm.): *Coenonympha pamphilus* (Linnaeus, 1758), *Polyommatus thersites* (Carterer, [1835]), and *Vanessa cardui*.

**Behaviour.** During the daytime investigation in the natural environment of *C. mystaphia*, it was discovered that larvae always feed on the underside of the leaves of *R. ribes*. Larvae move very slowly on the leaves and feed by grazing on the leaf surface, creating ‘windows’ on the

host plant leaves, except for the thick veins (Fig. 5) that are presumably more difficult to consume and digest, and lower in nutrients compared with newly-flushed apical leaves. Thus, they form many ‘windows’ of different sizes on plant leaves.

In rainy weather, the adult specimen shelters under the broad leaves of its host plant (O. Başbay, pers. comm. 2021) with wings being mostly closed. It was observed that adults seek nectar from the host plant’s flowers in preference to other sources. Adults were flying only short distances from *R. ribes* host plants. The butterfly is small, and has much the same colour as the leaves, making observation difficult; its habit of remaining in close proximity to the host plant most probably serves as a camouflage against predators. Thus, its potential to disperse is assumed to be limited, as it rarely strays more than a few metres away from the host plant.

**Parasitoids** (Fig. 6). During field studies at the Şirvan habitat on 1 June 2020, it was found that a fully-grown larva of *Callophrys mystaphia* had been attacked by a parasitoid. The parasitised larva continued its normal feeding on the first day of observations and turned yellowish colour. Then, its movements slowed and it became motionless, prior to the emergence of the parasitoid larvae and the subsequent formation of cocoons leading to larval death two days after formation of cocoons. The presence of cocoons alongside its body was found on 3 June 2020 (Figs 6a–c) and adult parasitoid wasps emerged on 9 June 2020. Male wasps emerged from two out of the five cocoons (Figs 6d–g). The other parasitoids could not emerge from the pupa due to fluid flowing from the dead larva.

From photographs, the parasitoid was confirmed by Donald Quicke and Mark Shaw (pers. comm. 2021) as a species of *Cotesia*, Cameron, 1981 (Hymenoptera, Braconidae, Microgastrinae). The Microgastrinae subfamily is the most significant group of parasitoid wasps that attack caterpillars, with many species being utilized or regarded as biocontrol agents against lepidopteran pests in agriculture and forestry (WHITFIELD 1997). The genus *Cotesia* is known to parasitize many species of butterflies. In addition, *Cotesia inducta* (Papp, 1973) is reported to parasitize *Callophrys rubi* (SHAW et al. 2009).

**Distribution** (Fig. 7). Formerly, *C. mystaphia* was claimed to be widespread in Turkey, Iran and Afghanistan (HEYDEMANN 1954; HESSELBARTH et al. 1995; TEN HAGEN 2006, 2010). However, it became clear that the populations in Iran and Afghanistan were not conspecific (for details see Introduction; KRUPITSKY et al. 2012, KRUPITSKY & KOLESNICHENKO 2013).

The species has been reported in 9 provinces from the east and south-east of Turkey according to the literature records: Kars (Kağızman) (MILLER 1913, HESSELBARTH et al. 1995), ?Aksaray (Ihlara), Iğdır (Aktaş) (HESSELBARTH et al. 1995; see below), Van (Erek Mt.) (KEMAL 2009), Siirt (Şirvan) (SEVEN 2010), Adıyaman, Kahramanmaraş (TSHIKOLOVETS 2011), Hakkari, and Tunceli (Ovacık) (KOÇAK & KEMAL 2018). It was also photographed by butterfly watchers from Bitlis (Ahlat), Elazığ, Gaziantep, Giresun, Kahramanmaraş, Malatya, Muş, Osmaniye, Siirt, Tunceli

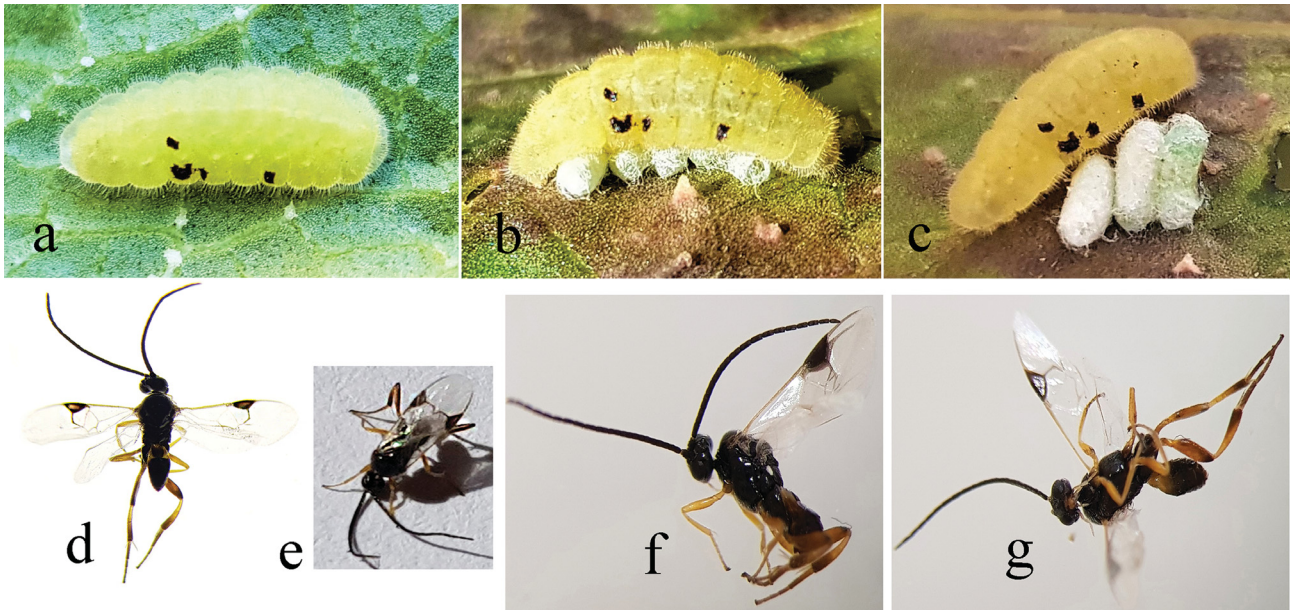


Fig. 6. a – Parasitised larva of *Callophrys mystaphia* Miller, 1913; b–c – cocoons of the parasitoid; c – intentionally removed larva to reveal the cocoons, d–g – reared parasitoids of the genus *Cotesia* (Braconidae: Microgastrinae) from various perspectives.

and Van Provinces (TRAKEL 2022).

*Callophrys mystaphia* has not been observed at the type locality and other previously published localities for many years (MILLER 1913, HESSELBARTH et al. 1995). After its first description in 1913, the species was figured from Kars (Kağızman) and Iğdır (Akteş) by HESSELBARTH et al. (1995). But they listed these samples as uncertain ('status incertus') because no adults could be found matching with the description and the lectotype (WAGENER & VAN OORSCHOT 1998). However, since *Rheum ribes* is known in these areas, it is believed that the species may still be present (KARAÇETİN & WELCH 2011). There were also unconfirmed specimens recorded from the valley of Ihlara (Aksaray) in HESSELBARTH et al. (1995). They had some doubts about whether the two males found by Wagener in 1985 at 1200 metres on a yellow-flowered Apiaceae in Aksaray (Ihlara Valley) were *C. mystaphia* or a different taxon. Moreover, there was some controversy concerning the diagnoses of the *Callophrys* species in Turkey when HESSELBARTH et al. (1995) released the study. *Callophrys paulae* was presented as a junior subjective synonym of *C. mystaphia* while WAGENER & VAN OORSCHOT (1998) were comparing the lectotype and the original description with material collected in the surrounding area of the type locality in June 1996 (WAGENER & VAN OORSCHOT 1998); therefore, they associated the habitat of *C. mystaphia* with *Onobrychis cornuta*, which is actually the habitat of *C. paulae*, and this result is based on a misleading taxonomical interpretation as they caught only *C. paulae* specimens near the type locality of *C. mystaphia*. Furthermore, the records of the sites in HESSELBARTH et al. (1995), the current observations (TRAKEL 2022) and the research reports (MILLER 1913, HESSELBARTH et al. 1995, KEMAL 2009, SEVEN 2010, TSHIKOLOVETS 2011, KOÇAK & KEMAL 2018) do not match exactly. Hence, the locations of the recorded species and food plant must be carefully established to determine the exact distribution of *C. mystaphia*. This

should be supported by extensive molecular studies of mitochondrial, nuclear, and ribosomal genes, including the populations in Turkey in case different taxa for this species may be present.

As indicated by KRUPITSKY & KOLESNICHENKO (2013), the status of isolated populations of butterflies similar to *C. mystaphia* inhabiting Adıyaman and Kahramanmaraş and cited in TEN HAGEN (2006) and TSHIKOLOVETS (2011) still remains controversial because of the likelihood that they belong to undescribed taxa. Additionally, a new plant species of rhubarb, *Rheum telianum*, was recently discovered in Adıyaman (IÇLİM & KARAHAN 2020). Therefore, Adıyaman's populations must be thoroughly examined, together with the other populations of the species in the south-east of Turkey, especially by means of detailed molecular analysis.

**Habitat** (Fig. 8). All recognized habitat types have much in common, largely consisting of eroded steppe with rhubarb plants. The habitat is given by TSHIKOLOVETS (2011), as 'mountain slopes and gorges with sparse vegetation and dominance of *Onobrychis cornuta* and *Rheum* spp. from 2000 up to 3000 m'. In the study area, *Callophrys mystaphia* inhabits mountainous and eroded stony steppe formations. Its vertical distribution ranges from 1400 m up to approx. 2800 m (HESSELBARTH et al. 1995, KARAÇETİN & WELCH 2011). The type material of *C. mystaphia* was caught at an altitude of about 1800 m (MILLER 1913). SEVEN (2010) reported this species between 950–1480 m in the Şirvan district of Siirt, and only two specimens were caught around 950 metres in Hesko and Maden junction; however, most of the adult samples were collected above 1380 m (see examined materials). *Rheum ribes* was not found in the Hesko and Maden junction locations. However, the presence of food plants in those habitats is undetermined because of the difficulty in accessing the steep slopes. Almost the entire research area in Şirvan (Siirt Province, SE Turkey) comprises mountainous terrain and has no



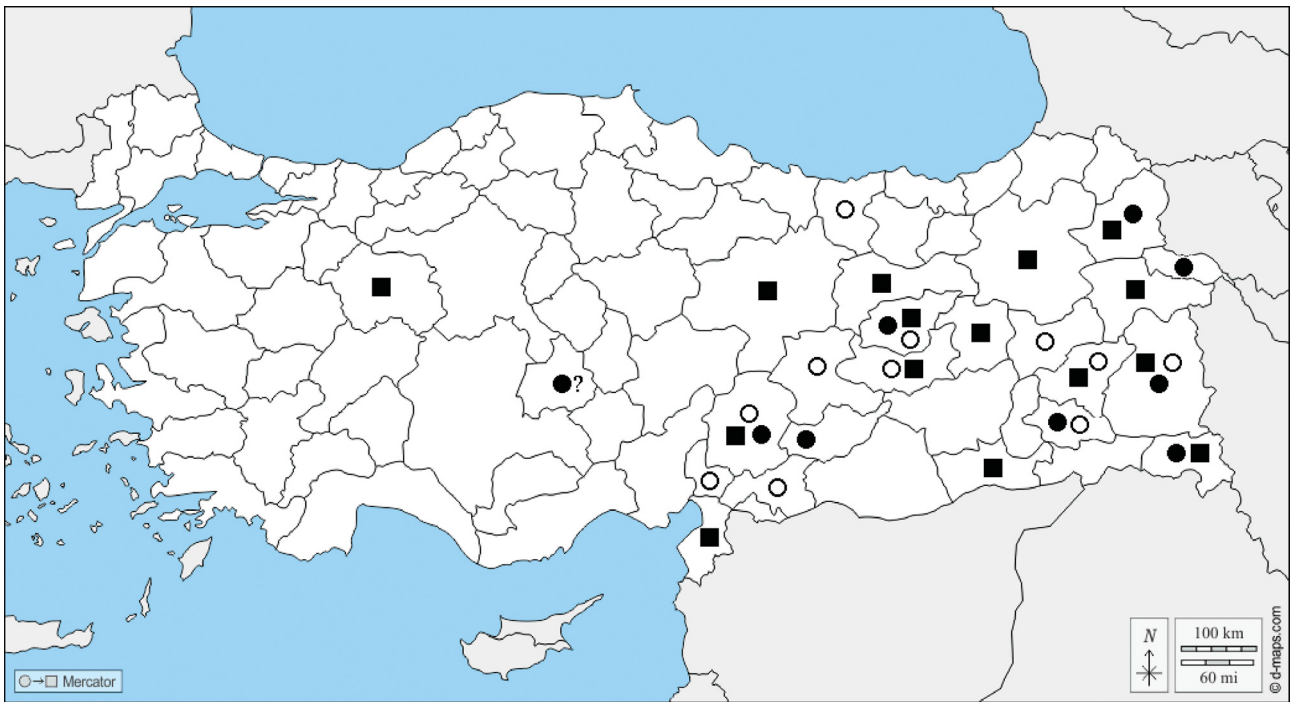


Fig. 7. Distribution map of *Callophrys mystaphia* Miller, 1913 (black circle: records based on scientific papers, empty circle: records of butterfly watchers) and *Rheum ribes* (square).



Fig. 8. Habitat of *Callophrys mystaphia* Miller, 1913 in Şirvan district, Siirt Prov., south-eastern Turkey, 1400 m a.s.l. (a – 1.vi.2020, b – 29.iv.2021).

significant wide valleys or plains. The district is divided by the Kezer stream in the west and the Botan River in the east. In the south, the vegetation is largely steppe, whereas in the north, stands of oak are concentrated in mountainous regions. After a rather short spring, precipitation reduces, and the hot, dry air flow from Basra (Iraq) continues until October. Herbaceous plants outside the stream border begin to dry out in early June, leaving practically solely woody and bushy plants (SEVEN 2014). The deep gorges of Botan, Harbur, and Zab in Southeast Anatolia, south of Van Lake, along with their rich vertical structures, provide a favourable refuge for various tertiary relicts and elements of the Turk-Iranian Zagros fauna and flora, which are not found in other Turkish locations (WAGENER 2005).

Maden road's location contains herbaceous plant and small shrub species such as *Cirsium*, *Gundelia* (both Asteraceae), *Astragalus*, *Medicago*, *Onobrychis*, *Trifolium*, and *Vicia* (all Fabaceae), which are common in spring. Sparsely found woody plants in the area are *Quercus* (Fagaceae), *Salix*, *Populus* (both Salicaceae), *Crataegus*, *Rubus* (both

Rosaceae), and *Paliurus spina-christi* and *Rhamnus* (both Rhamnaceae). The habitats in Kahramanmaraş and Bitlis (Ahlat) Provinces are defined as barren, steppe, mostly dominated by *R. ribes* (O. Başbay, pers. comm. 2021).

**Host plant.** Polygonaceae: *Rheum ribes* L. (KEMAL 2009, this paper).

*Prangos lophoptera* Boiss. (Apiaceae) was mentioned as a possible host plant in the original description by MILLER (1913). WAGENER & VAN OORSCHOT (1998) specified that *C. mystaphia* was linked with the subalpine thorn-cushion plant formation associated with *Onobrychis cornuta* (L.) (Fabaceae). However, larvae were found on *Rheum ribes* L. (Polygonaceae) in Ereğ Mt., Van Prov. of Turkey (KEMAL 2009). The species is also determined as feeding on *R. ribes* in Siirt Prov., in the research area, and under laboratory conditions. Additionally, Iranian *C. mystaphioides* was stated to be associated with *Rheum* sp. in known localities – both in the type locality in Esfahan Province as well as in Fars Province (Iran). *Rheum persicum* is considered a potential host plant of *C. mystaphioides*

(TEN HAGEN 2006). Furthermore, *C. titanus* Zhdanko, 1998 uses *Rheum* sp. as host plant in Tajikistan (ZHDANKO 1998). As in the species mentioned above, *Rheum* spp. is the only known food plant for some closely related species of this genus. On the other hand, *C. rubi* is extremely polyphagous and feeds on a large variety of plants from about 10–15 different plant families (FIEDLER 1990).

*Rheum ribes* L. (in Turkish: “İşgin”) is an edible wild species of rhubarb and is an element of the Iran-Turan Phytogeographic Region ranging from Israel, Palestine, Lebanon, Armenia, and Northern Iraq to Iran and the Eastern Anatolian Region of Turkey (CULLEN 1996). In Turkey it is reported in the following areas: Ağrı (Tahir-Eleşkirt, Kavurman Mt.), Bingöl (Çirişli Pass, Kop Mt., Bingöl-Erzurum provinces border), Bitlis (Ahlat, Kambos Mt.), Eskişehir (Anadolu University Campus), Elazığ (Harput, Kup and Mastar Mts., Pekinik), Erzincan (Keşiş Mt., Guleman), Erzurum (İlica), Hakkari (Otluca, Sat Mt.), Hatay (İskenderun, Arsuz Amanos Mt.), Kahramanmaraş (Göksun-Geben Kayranlı Mt.), Kars, Mardin (Hessena), Sivas, Van (Erek Mt., Değirmenköy, Gölardı, Bahçesaray, Gürpınar), Tunceli (Munzur Mt., Ovacık) (CULLEN 1966, MUNZUROĞLU et al. 2000, KOROTYAEV et al. 2016, IÇLİM & KARAHAN 2020, AKIN & KURŞAT 2021).

The provinces of Ağrı, Bingöl, Eskişehir, Erzurum, Erzincan, Hatay, Mardin, and Sivas are areas where the food plant is known but *C. mystaphia* is yet to be found there, as it is most likely present. The population status, distribution, and current distribution limits of the butterfly species need to be confirmed by investigating all the locations where the host plant is documented (Fig. 7). Studies on the current subject are still in progress.

*Rheum ribes* is reported to be a food source for a variety of insects. Noctuid moth *Xylena exsoleta* (Linnaeus, 1758) feeds on *R. ribes* leaves (KEMAL et al. 2008), and *Apochima diaphanaria rjabovi* (Wehrli, 1936) (Geometridae) on the blooms (AKIN & KURŞAT 2021). Also, weevil *Petrocladus* sp. (Curculionidae), jewel beetle *Capnodis marquardtii* Reitter, 1913 (Buprestidae), and leaf beetle *Labidostomis brevipennis* Faldermann, 1837 (Chrysomelidae) are insects associated with *R. ribes* in eastern Turkey (KOROTYAEV et al. 2016). Moreover, hymenopter species *Kokujewia ectrapela* Konow, 1902 (Argidae) feeds alongside *C. mystaphia* on *R. ribes* leaves in Şirvan district (SEVEN 2022). These are all plant-specific herbivores, and the majority appear to be native to Turkey. Additionally, it was observed that the larvae of some other insect taxa from different groups (such as Hymenoptera, Coleoptera and Noctuidae) also feed with *C. mystaphia* on *R. ribes* in the field. Further research is pending, and is hoped to be reported upon later. **Conservation status.** *Callophrys mystaphia* is a small, local and monophagous species and probably one of the scarcest butterflies in Turkey (KEMAL 2009). It generally prefers to live around the host plant, making only short flights, and its distribution is thus limited by the presence of food plants in Turkey. The species is endangered due to the restricted distribution and pressure on the food plant (KARAÇETİN & WELCH 2011).

The larval food plant, *Rheum ribes* L. (Polygonaceae) is a perennial herbaceous wild plant species and has medicinal importance (BAYTOP 1999). In traditional Anatolian folk medicine, extracts from various parts of the plant are commonly used to treat a variety of diseases (YILDIRIM et al. 2020). Since *R. ribes* roots are considered to be beneficial for the treatment of diabetes, they are dug up by the local people (SEVEN 2010). Collection and sale of food plants as vegetables in local markets are uncontrolled, and young shoots are consumed fresh by the public. However, excessive picking of food crops, habitat loss, and plant destruction due to consumption and overgrazing are some of the most crucial threats to the species, and these may lead to the extinction of *R. ribes*, not a very common plant with a highly specific assemblage of insects, most of which are endemic to Turkey (KOROTYAEV et al. 2016). Therefore, it is essential to preserve the long-term sustainability of *R. ribes* harvest, while at the same time maintain control over collection and marketing activities.

The species' records are restricted, as the duration of its flight period is short, the number of the populations is low, and investigating the host plant, which grows on steep stony slopes at high altitudes, is challenging. Primarily, it may be easier to identify the food plant to determine the possible locations of the butterfly species. However, this may not be satisfactory because the low butterfly population is commonly concentrated on small numbers of plants and sites. The study region, Maden road location, is estimated to have one of the largest populations of the species. Population reports were extremely scarce in almost all other habitats in Turkey (TRAKEL 2022). Also, the populations in Kahramanmaraş and Bitlis provinces are stated to be rare (O. Başbay, pers. comm. 2021).

KARAÇETİN & WELCH (2011) described *C. mystaphia* as ‘near endemic’, taking into account the populations in eastern Turkey and southwestern Iran. However, the population in southwestern Iran was afterwards described as a different species (KRUPITSKY & KOLESNICHENKO 2013). As *C. mystaphia* occurs only in a restricted region with small local populations and has a short flying time, the species is thought to be endangered. *Callophrys mystaphia* was included in “Lycaenidae protected by legislation in Europe” (COLLINS 1987). Taking into account all of these factors, it is recommended that the species and its only host plant, *R. ribes*, should be protected.

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