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A new species of *Contarinia* (Diptera: Cecidomyiidae) from flower galls on the relict tree *Zelkova abelicea* (Ulmaceae) endemic to Crete (Greece)

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Abstract

Contarinia ampelitsiae **n. sp.** Dorchin & Fazan is described as a newly discovered gall-midge species (Diptera: Cecidoymiidae) forming galls in flowers of *Zelkova abelicea* (Ulmaceae), a tree species endemic to the Mediterranean island of Crete (Greece). Larvae develop within modified filaments of male flowers, contrary to many *Contarinia* species that develop freely in flowers or in simple flower galls. The species has one generation per year, and its galls are sometimes found in great numbers on individual trees, thus affecting both fruit quantity and weight. This is the first report of a gall midge from *Zelkova* and the first record of *Contarinia* from Ulmaceae. Based on its host-plant association and on the barcoding section of the mtCOI gene, this species has no obvious relatives within *Contarinia*.

Key words: Cecidomyiini, gall midge, Mediterranean, stamen filaments

Introduction

The genus *Zelkova* (Ulmaceae) is a climate relict that was widespread throughout the Northern Hemisphere during most of the Paleogene (Burnham 1986; Zhang *et al*. 2017). The genus is now restricted to three main disjunct areas and includes 6 extant species (Wang *et al*. 2001). *Zelkova serrata* (Thun.) Makino*, Z. sinica* C.K. Schneid. and *Z. schneideriana* Hand.-Mazz. are found in south-eastern Asia, *Z. carpinifolia* (Pall.) K. Koch is found in the Transcaucasian and Middle East regions, while the remaining two species are endemic to Mediterranean islands: *Z. sicula* Di Pasq., Garfì & Quézel in Sicily (Italy) and *Z. abelicea* (Lam.) Boiss. in Crete (Greece). *Zelkova abelicea* grows between 800 and 1800 m a.s.l. in all five mountainous areas of Crete, forming scattered and isolated stands (Egli 1997; Fazan *et al.* 2012). The species is ranked as endangered on the IUCN Red List (Kozlowski *et al*. 2012), and unsustainable pastoral practices are the most important threat, alongside changing climatic conditions and summer drought (Egli 1995; Fazan *et al*. 2012; Fournaraki & Thanos 2006; Kozlowski *et al*. 2018; Søndergaard &

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Egli 2006). A large majority of individuals exhibits a stunted dwarfed morphology and a very slow growth rate due to severe overbrowsing by domesticated goats (Fazan *et al*. 2012). These individuals can reach several centuries in age and cannot produce fruit unless they grow tall enough to escape browsing pressure (Fazan *et al*. 2012; Kozlowski *et al*. 2012). Arborescent, 15–20 m tall individuals are much rarer and are estimated to represent only ca. 5% of all individuals (Kozlowski *et al*. 2012). These arborescent individuals experience masting events every 2–3 years, during which most fruiting trees produce fruit synchronously, while only few trees fructify, on relatively few branches, in the in-between years (Fazan *et al*. 2023; Egli 1997; Fournaraki & Thanos 2006; Søndergaard & Egli 2006). Trees in the genus *Zelkova* are monoecious (Denk & Grimm 2005), and flowers are produced on specialized flowering shoots with a clear zonation of flower sex (Fig. 1) such that clusters of caducous male flowers are found on the most basal part of the shoots, hermaphrodite flowers are intermediate and solitary female flowers are produced at the top of the shoots (see Fazan *et al*. 2023 for more details). These specialized shoots act as dispersal units in autumn, when they abscise at the base and disperse together with dried leaves and fruit through wind (anemochory) and gravity (barochory) (Fazan *et al*. 2023; Egli 1997; Hoshino 1990; Oyama *et al*. 2018).

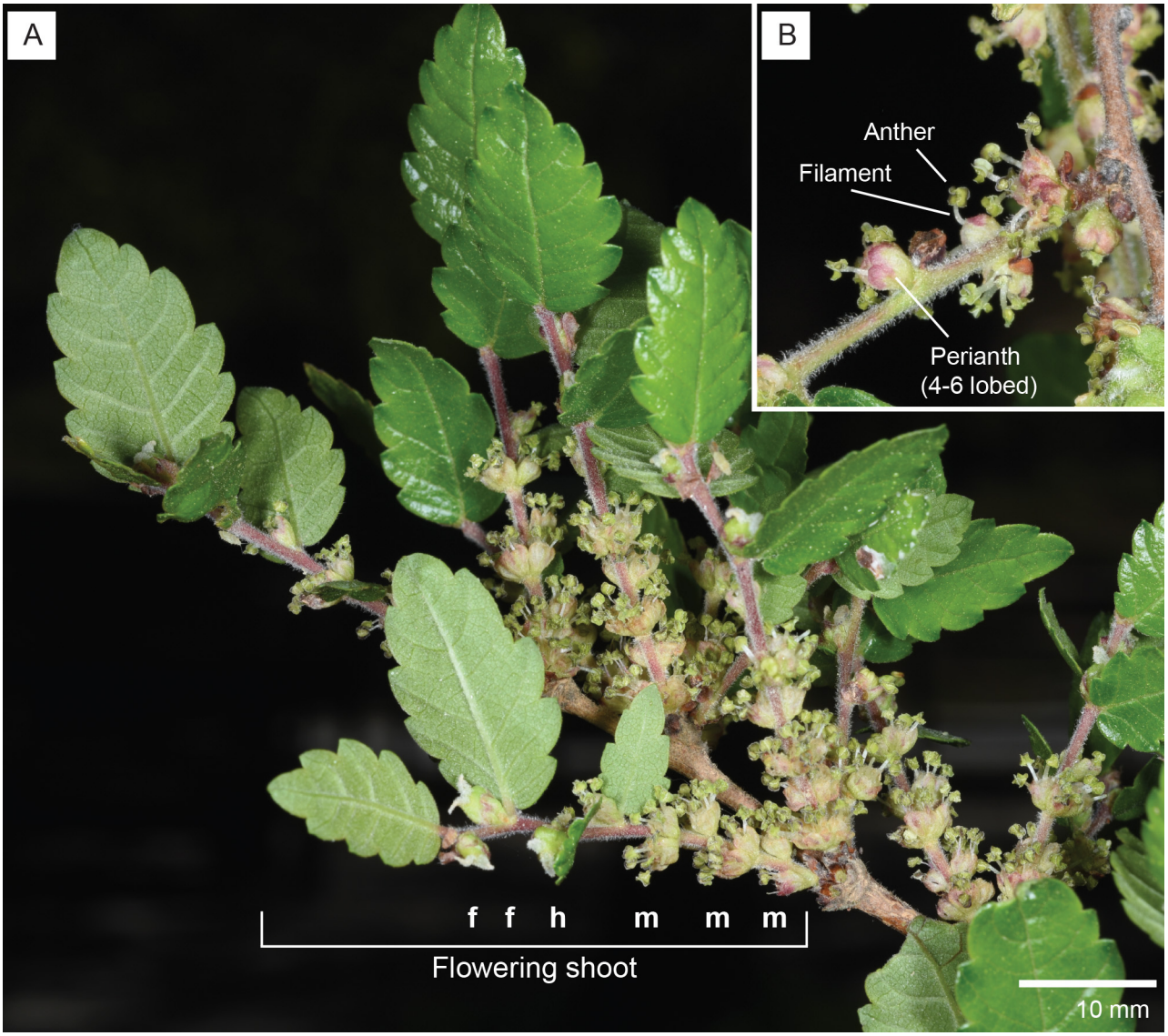


FIGURE 1. A. Flowering shoot of *Zelkova abelicea* with a clear zonation of flower sex, with proximal male flowers (m), intermediate hermaphrodite flowers (h) and distal female flowers (f). B. Morphology of male *Z. abelicea* flower. Each male flower is composed of 4–6 stamens, each with a filament and anther and surrounded by a 4–6 lobed perianth.

In 2017, we first noticed from photographic evidence and then confirmed in the field that at several localities across Crete, the male flowers of *Z. abelicea* are galled in spring by an unknown insect, which was subsequently found to be an undescribed species of gall midge from the tribe Cecidomyiini. Gall midges constitute the largest

group of gall-inducing insects, and many make galls on plants belonging to diverse plant families worldwide (Gagné 1989; Gagné & Jaschhof 2021). Nevertheless, only 8 gall-midge species are currently known from Ulmaceae, all on *Ulmus* spp., and none of these belongs to the Cecidomyiini (Gagné & Jaschhof 2021). Here, we describe the newly discovered species from *Z. abelicea* flowers as new to science based on its adults, larvae, and galls, provide information about its life history and discuss its possible impact on the ecology of its host plant.

Material and methods

Mature galls were collected from two *Z. abelicea* trees in the following localities in Crete, Greece: 27 May 2020, Omalos Plateau, district of Chania, 35.318843 N, 23.913969 E, and 28 May 2020, Katharo Plateau, district of Lassithi, Crete, Greece, 35.144263 N, 25.573718 E. The galls were placed in plastic vials containing potting soil and covered individually by fine-scale mesh cloth before being sent by rapid postal service to the University of Fribourg in Switzerland. Each plastic vial was then transferred into a larger pot containing potting soil, and each pot was covered individually in fine-meshed cloth, hermetically closed and kept in an outdoor, shaded location. The pots were regularly watered in order to keep the soil moist, and regularly monitored in order to check for emerging insects. Emerging adults as well as larvae excised from the galls were preserved in 70% ethanol for morphological study and were later mounted on permanent microscope slides in Euparal following the method described in Gagné (1994). One adult was used for sequencing the "barcoding" section of the mitochondrial COI gene according to the methods described in Dorchin *et al*. (2019). Terminology for adult morphology follows Cumming and Wood (2009), while terminology for immature morphology follows Gagné (1989). Illustrations of morphological characters were made with a drawing tube attached to a Leica DM100 LED compound microscope. Galls and types are deposited in the Natural History Museum of Fribourg (NHMF) and in the Steinhardt Museum of Natural History, Tel Aviv University (SMNHTAU) as specified below in the species description. Acronyms for the herbaria in which dry plant specimens are deposited follow Thiers & Ramirez (2021).

Results and discussion

The newly described species belongs to *Contarinia* Rondani 1860, one of the biggest, loosely defined and paraphyletic genera of Cecidomyiidae, with 318 described species worldwide (Gagné et al. 2018; Gagné & Jaschhof 2021). Many species of this genus develop in simple flower galls, mostly as gregarious larvae in unopened and slightly to conspicuously deformed flowers on host plants belonging to many different families (e.g., Gagné 1989, Fedotova 1991, 1993, 1994; Uechi *et al*. 2011; Kolesik *et al*. 2017; Mori *et al*. 2019). The overwhelming majority of species leave their galls to pupate in the soil; many of them complete several generations per year, but some are bivoltine or univoltine.

Galls of the species described here were found on fruiting *Z. abelicea* trees in Crete (Greece) in spring 2017, and subsequent investigations revealed the presence of galls on some (not necessarily all) fruiting trees from several localities throughout the host-plant range, i.e. Omalos, Therisso, Ano Meros and Katharo. No galls were noticed on trees from Impros, Gerakari, Rouvas, Omalos Viannou and Protolitsa. The prevalence of the galls was highly variable according to locality and tree. Some trees had enormous quantities of galls that were visible even from a distance (e.g. Fig. 2A), others had only a few galls per fruiting shoot (e.g., Fig. 2B), while others had no galls at all. Fazan *et al*. (2023) recorded up to 13 galls per fruiting shoot with an average (for galled shoots) of 3 galls per shoot. This gall-midge species has one generation per year; galls form in mid-April to early May, in individual filaments of stamens on male or hermaphrodite flowers (Figs 3–4). On heavily galled trees, galls may also be exceptionally found on leaf blades (L. Fazan, pers. obs.). The filaments are deformed, swollen, enlarged, change color and become persistent while the anthers acquire a lateral position (Fig. 3). The normally whitish-cream colored and glabrous filaments become green to reddish and covered in hairs in fresh galls. The gall is conical, its terminal part is curved inwards and contains an opening lined with hair, through which the larva exits when mature (Fig. 3). More than one filament per flower can be galled. In hermaphrodite flowers, the upper tissues of the female flower part (and then fruit pericarp) become fused with the gall (Fig. 4). Each gall usually contains a single larva. Mature larvae leave the gall, drop to the ground and burrow in the soil, where they stay dormant from the onset of summer (late May or early June) until the end of

winter. Once the larvae leave the galls, the galls dry out and become brown but persist on the tree and are still found on dispersal shoots at the time of fruit dispersal in autumn (Fig. 2). Adults emerge in spring.

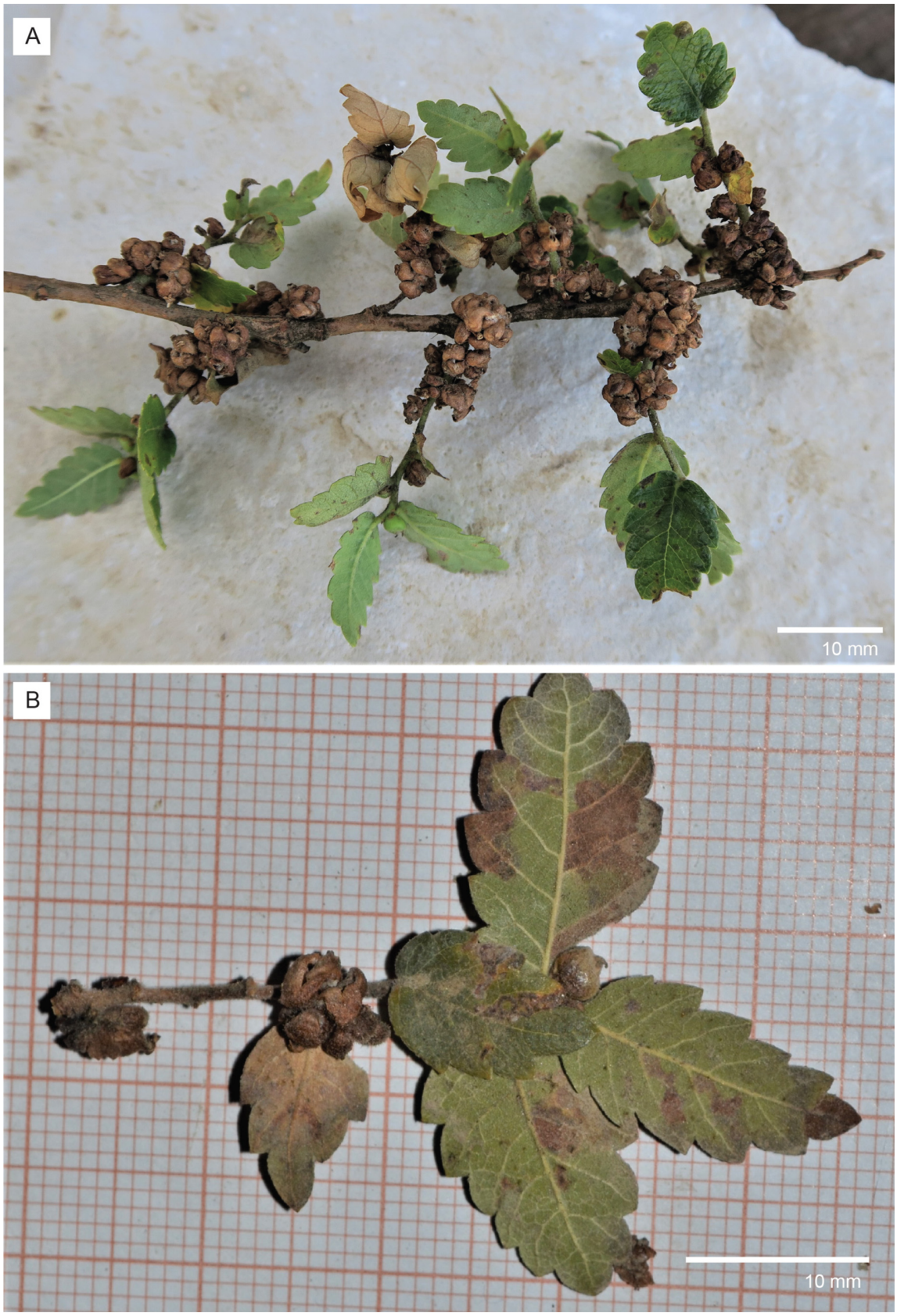


FIGURE 2. A. *Zelkova abelicea* branch composed of several strongly galled flowering shoots. After the exit of the larvae, galls become brown and dry out. B. Galls persist on the flowering shoots until autumn and can be found attached to the dispersal unit (i.e. flowering shoot).

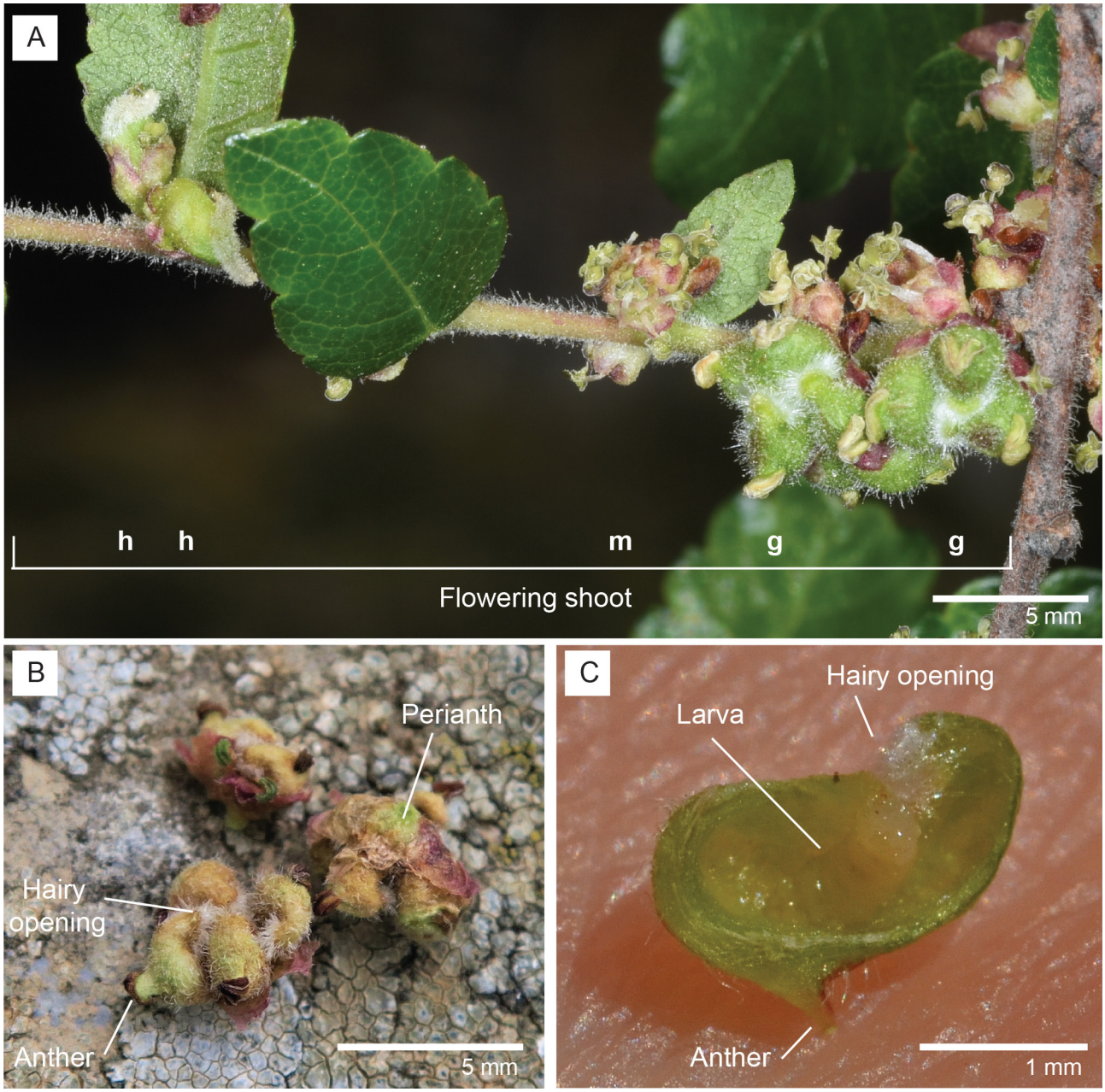


FIGURE 3. A. Flowering shoot of *Zelkova abelicea* with galled male flowers (g), non-galled male flowers (m) and hermaphrodite (h) flowers. Galls are formed on individual stamens. B. Details of galled male flowers. The anther becomes lateral and there is an opening at the tip of each gall, covered in hair. C. Cut gall with larva.

In this study, we managed to rear 8 adults (Fig. 5) that emerged between 31st March and 2nd April 2021 from pots containing larvae only from the sampled tree in Katharo. This low survival rate and the failure to rear adults from samples collected in Omalos may be largely due to high parasitism rates, given that we reared numerous individuals of 3 taxa of parasitoid Hymenoptera: 48 individuals of *Aprostocetus* sp. (Eulophidae) from Omalos reared in June 2020 and 2021, 54 individuals of *Gastrancistrus* sp. (Pteromalidae) from Omalos and Katharo between November 2020 and January 2021, and 90 individuals of *Inostemma* sp. (Platygastridae) from Omalos between late March and end of April 2021.

Contarinia ampelitsiae Dorchin & Fazan, new species

Diagnosis. This species is placed in *Contarinia* for the binodal male flagellomeres, each with a single whorl of circumfila, untoothed tarsal claws, long, retractable and tapered ovipositor with greatly reduced, closely juxtaposed cerci, and 4 pairs of larval terminal papillae, two of which are coniform and recurved. The species has no obvious relatives within *Contarinia*, given that it forms previously unknown, elaborate stamen galls on Ulmaceae, a plant family from which no *Contarinia* species are known. No species of *Contarinia* are known also from the closely related plant family Cannabaceae, formerly included in the Ulmaceae, from which numerous cecidomyiid species of other genera are known (Gagné *et al.* 2013). Moreover, *Contarinia ampelitsiae* is univoltine, which excludes the possibility that it develops on other host plants. Adults have no dorsal occipital protuberance, the female antennal flagellomeres have very short necks, male antennal flagellomeres with necks as long as nodes, and larva with long-shafted, bilobed spatula.

Description.

Adult. *Head* (Fig. 6)*:* Eye facets round, more spacious laterally than medially. Occiput without dorsal protuberance; only slightly raised towards shallow, blunt projection carrying two long apical setae (Fig. 6). Frons with 4–5 long setae on each side. Labellum tapered from wide base, with several subapical setae. Palp four segmented, segments successively longer, palpiger present (Fig. 6). Male antennal flagellomeres (Fig. 7) with necks about same length as nodes, slightly longer in distal than in proximal flagellomeres; circumfilar loops reaching base of next node or slightly longer. Female flagellomeres (Fig. 8) with very short necks, each with two whorls of appressed circumfila connected by one longitudinal band.

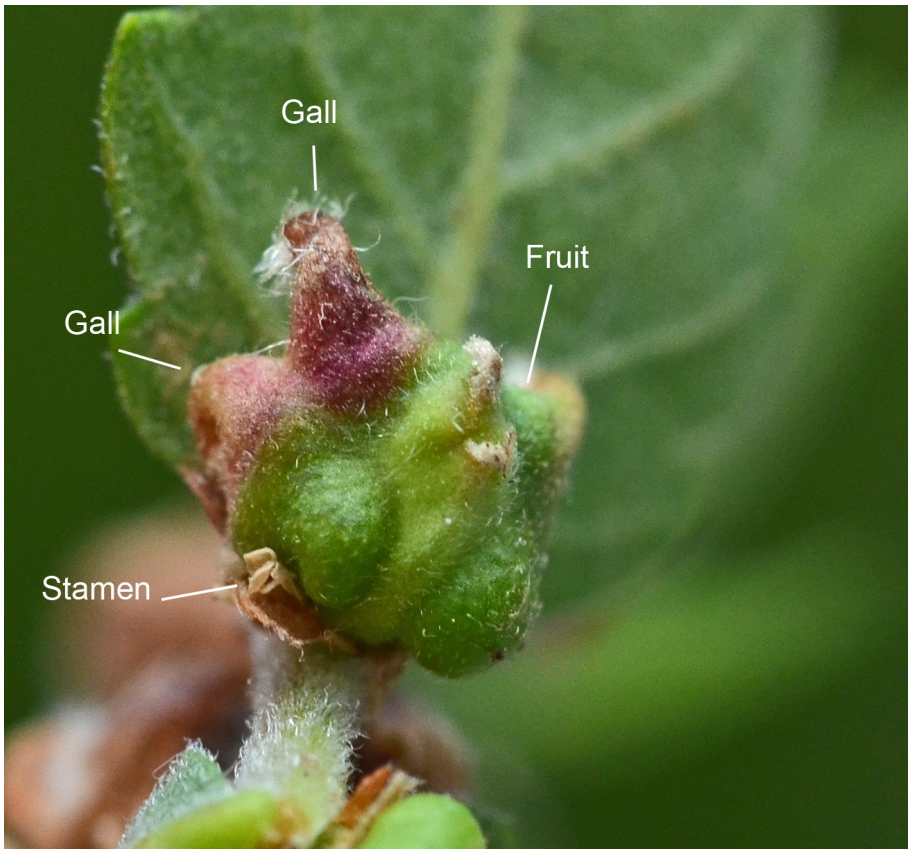


FIGURE 4. Hermaphrodite *Z. abelicea* flower with galls. The galls fuse with the external tissues of the fruit. Here two stamens were galled while one was not.

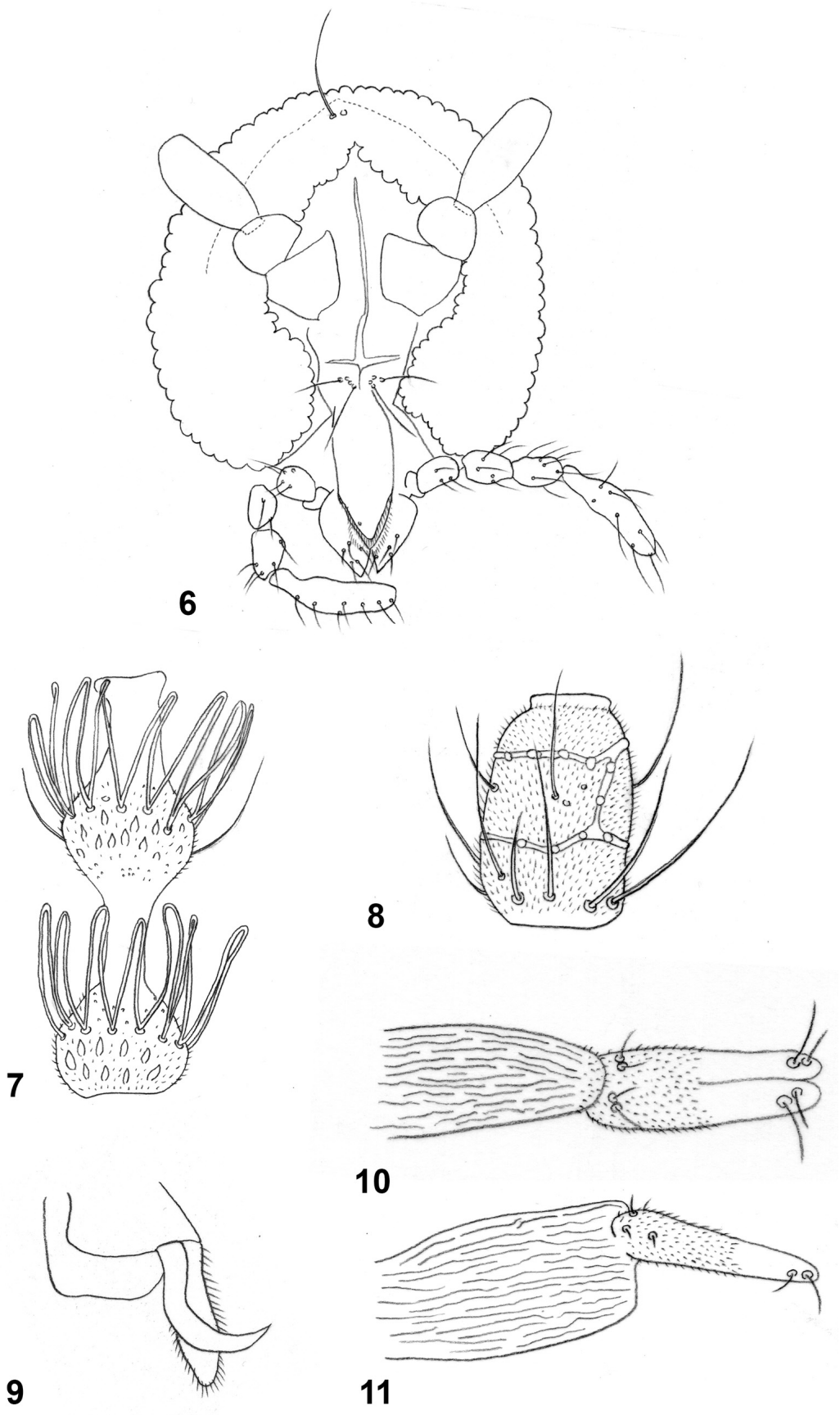
Thorax: Wing transparent, with long, fine, hair-like setae along posterior margin, R_{4+5} slightly bent, reaching C beyond wing apex. C with small break immediately past juncture with R_{4+5}. Wing length 2.15–2.56 mm in females (n=3), 1.93–2.34 mm in males (n=4). Legs covered by long, narrow scales; tarsal claws (Fig. 9) untoothed, curved along distal half, empodia longer than bend in claw, pulvilli minute.

FIGURE 5. *Contarinia ampelitsiae*. Male.

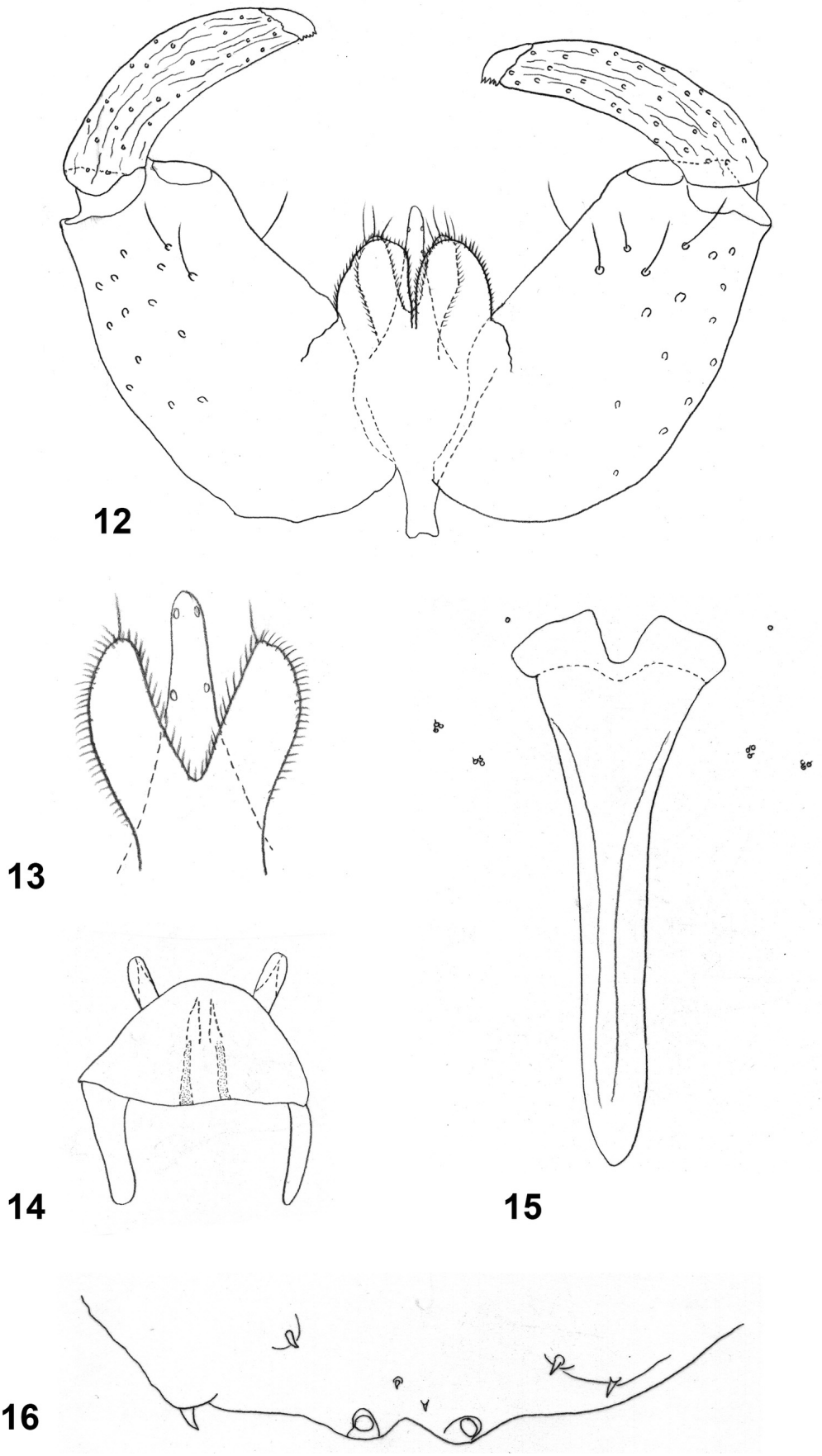
Female abdomen: Tergites 1–7 with anterior pair of sensory setae, posterior row of setae, and a group of 5–6 baso-lateral setae. Tergite 8 smaller than preceding, with few posterior setae. Sternites 2–7 with anterior pair of closely approximated sensory setae, continuous posterior row of setae and numerous evenly distributed setae laterally; pigmentation of sternites broken along thin line just proximal to posterior row of setae; sternite 8 undifferentiated from surrounding tissue. Ovipositor 10.7–12.3 as long as tergite 7 (n=3). Cerci (Figs. 10–11) closely appressed, about 4 times as long as wide, each bearing two strong setae apically and two smaller setae basally, microtrichose along proximal half, bare thereafter.

Male abdomen: Tergites 1–6 as in female; tergite 7 with only 2–3 lateral setae and without posterior row of setae; tergite 8 with much smaller sclerotized area, anterior pair of sensory setae the only vestiture. Sternites 2–6 as in female; sternites 7–8 without clear separation between lateral and posterior setae. Terminalia (Figs. 12–13): Gonocoxites robust, cylindrical, with evenly distributed long setae; gonostyli about same width throughout length, only slightly narrowed towards apical tooth, entirely glabrous and carinate, with evenly distributed setae; cerci separate to base, with several apical setae; hypoproct deeply divided into two cylindrical lobes, strongly microtrichose, each with one long seta apically (Fig. 13); aedeagus longer than hypoproct, narrow, tapered to rounded apex, with two pairs of asetose sensory organs on each side.

Larva (third instar) (Fig. 3C): Length 3.17–3.81 mm (n=10), whitish to pale yellow; antenna about 1.5 times as long as wide, cephalic apodemes as long as head capsule (Fig. 14). Spatula (Fig. 15) long shafted, with two wide, laterally rounded, mesally slightly pointed lobes separated by deep depression; broadest at base of lobes. On each side of spatula two triplets of tiny papillae, two in each triplet with barely visible setae. Third thoracic segment and first to eighth abdominal segments each with ventral field of minute spicules arranged in 6–15 transverse rows on mid-anterior section of segment; integument otherwise smooth. Coniform terminal papillae recurved, other three pairs with short, thick setae on slightly elevated bases (Fig. 16).



FIGURES 6–11. *Contarinia ampelitsiae*. 6. Head (only one apical seta shown). 7. Male 4th antennal flagellomere. 8. Female 4th antennal flagellomere. 9. Acropod. 10. Female cerci, dorsal. 11. Female cerci, lateral.



FIGURES 12–16. *Contarinia ampelitsiae*, 12. Male terminalia, dorsal 13. Male hypoproct and aedeagus, dorsal. 14.. Larva, head. 15. Larva, spatula and associated papillae. 16. Larva, terminal papillae.

Pupa (based on exuviae): A few degraded exuviae enabled only partial description. Antennal bases form very small and short tapered tips. Cephalic seta long and thin.

Etymology. The specific name refers to the local Cretan name for *Z. abelicea*, namely ampelitsiá (αμπελιτσιά).

DNA: The barcoding section of the mitochondrial COI gene sequenced from one male of *C. ampelitsiae* was deposited in GenBank under accession number OQ107473. Comparison of this sequence to available sequences in GenBank showed highest percentage identity of 91.03–91.73% with numerous unidentified cecidomyiid specimens, with the highest similarity to other *Contarinia* species being 91.18% to *Contarinia caryafloralis* from *Carya* in China (accession MH039840.1, Jiao *et al*. 2018), and 90.66% to an unidentified *Contarinia* species from *Oxalis* in Japan (accession AB597022.1, Uechi *et al*. 2011). These values indicate that *Contarinia* species for which relevant sequences are currently available in GenBank are very different from *C. ampelitsiae* and are unrelated to it.

Material examined: *Holotype***:** ♀, Greece, Crete, Katharo plateau, 35.144263 N, 25.573718 E, 28.v.2020, I. Remoundou. Ex stamen galls of *Zelkova abelicea*. Emerged on 31.iii.2021. On permanent microscope slide in Euparal. Deposited in NHMF (accession GBIFCH01352024). *Paratypes***:** All from Greece, Crete, Katharo plateau, 35.144263 N, 25.573718 E, 28.v.2020, I. Remoundou. 10 larvae (on 5 microscope slides, 6 NHMF: accessions GBIFCH01352028, GBIFCH01352029, GBIFCH01352030, 4 SMNHTAU: accessions 427728, 427729); 2♂, 1♀ (NHMF: accession GBIFCH01352025, GBIFCH01352026, GBIFCH01352027); 2♂ 1♀, (SMNHTAU: accessions 427726, 427727, 416127), emerged between 31.iii.2021 and 02.iv.2021. *Other material*: 3 pupal exuviae on two microscope slides, Greece, Crete, Katharo plateau, 35.144263 N, 25.573718 E, collected on 28.v.2020 by I. Remoundou; excised from soil on 11.xi.2021 by L. Fazan (SMNHTAU).

Comments. The first and only previous known mention, description and drawing of the galls of this insect come from an unsigned and undated note in French inserted in the collection of the Herbarium of the University of Montpellier (MPU) (Supplementary material S1). The note is accompanied by a few flowering shoots of *Z. abelicea*, with leaves, fruit and galls contained in an envelope. This note is thought to have been written by the French botanist Jules Émile Planchon (1823–1888), and mentions that the galls come from *Z. abelicea* samples collected by the German botanist Theodor von Heldreich (1822–1902), giving a morphological description of the galls and stating that no insects were found within. No samples collected by von Heldreich are currently included in the herbarium of MPU, but samples collected by him in June 1846 which contain galls are found in the collections of other herbaria, e.g., the Conservatoire et Jardin botaniques de la Ville de Genève (G), the Muséum national d'Histoire naturelle of Paris (P), the Royal Botanic Garden of Edinburgh (RBGE), and the University of Florence (FI). J. E. Planchon included his observations of the galls in his description of *Z. abelicea* found in the *Prodromus Systematis Naturalis Regni Vegetabilis* of de Candolle (Planchon 1873).

Recent studies (Fazan *et al*. 2023) have shown that the presence of *C. ampelitsiae* on flowering shoots of *Z. abelicea* significantly reduces the number of fruits produced per shoot and fruit weight, probably due to shifts in resource allocation. Seed sterility issues are of major concern over parts of the range of *Z. abelicea*. However, no significant effect of the presence of *C. ampelitsiae* on the overall seed sterility of the trees has been found.

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