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# Three leafhoppers newly recorded from the European mainland (Hemiptera: Auchenorrhyncha: Cicadellidae), with notes on their habitats

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#### ABSTRACT

The leafhopper fauna of the Iberian Peninsula is the least studied in Europe. Recent surveys in agroecosystem areas of southern Spain, specifically in Andalusia, aimed to identify potential Auchenorrhyncha vectors of the bacterium *Xylella fastidiosa*. During these surveys, three previously unrecorded species of Cicadellidae belonging to the subfamily Deltocephalinae have been discovered. These newly recorded species are *Balclutha incisa* (Matsumura), *Cicadulina* (*Cicadulina*) *bipunctata* (Melichar), and *Maiestas angustisecta* (Linnavuori). Generally, these species were previously only known from American, African and eastern Palaearctic regions. This work is the first to record these species on the European mainland. Detailed descriptions of adult specimens have been provided, along with information on their current distribution and host plants. The potential role of these leafhoppers as potential vectors of plant pathogens is discussed, highlighting their importance in agroecosystems.

Key words: Macrostelini, Deltocephalini, Southern Spain, vectors, distribution

## **INTRODUCTION**

Auchenorrhyncha (Hemiptera) is a diverse group of plant sap-sucking herbivores, consisting of approximately 47,330 species worldwide (Dmitriev *et al.* 2022). They are divided into two infraorders: Fulgoromorpha and Cicadomorpha. The latter, with around 4,220 genera and 22 families, is the most diverse. Cicadellidae is the largest family, comprising 23 subfamilies and about 23,181 distinct species. These insects are of significant importance as agricultural pests and disease vectors and play a crucial role in terrestrial ecosystems, being present in almost all environments where vascular plants grow (Dietrich 2013; Mifsud *et al.* 2010; Nielson 1968). Despite their ecological and economic significance, the Auchenorrhyncha of Spain have been poorly studied. Most Spanish regions lack comprehensive or reliable information, with few regional checklists available (Aguin-Pombo *et al.* 2007, 2008; Pérez Hidalgo *et al.* 2015; Pérez-Gómez *et al.* 2020). This is particularly true for Andalusia, the southern region of Spain, which boasts a high insect diversity (García-Barros *et al.* 2002; Puissant & Sueur 2010). However, knowledge of Auchenorrhyncha in this region is outdated, biased towards economically important species, or information is scattered across entomological literature (Durán Alvaro 2018; Morente *et al.* 2018; Pérez-Gómez *et al.* 2020; Serrano Caballos *et al.* 2016).

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Alien species can have significant ecological, economic, and social consequences for the environment. These introductions pose a threat to diversity, agriculture and human health (Rojas-Sandoval *et al.* 2017; Roques *et al.* 2009). Factors such as global commerce, transportation, and human disturbance contribute to the spread of alien species (Gilardi *et al.* 2018; Morand & Lajaunie 2021). Specifically in the agricultural sector, not only can they cause direct damage as new pests, but they can also lead to outbreaks of emerging and reemerging diseases (della Giustina 1989; Wilson & Weintraub 2007). A recent example of this is the 'rapid decline syndrome of olive trees' in Italy, which is caused by the introduction of the bacterium *Xylella fastidiosa* Wells *et al.* (Saponari *et al.* 2013), highlights the potential consequences of alien species and the need for effective measures to prevent their spread. Regarding the Auchenorrhyncha group, D'Urso *et al.* (2019) report that there are 30 alien species in Europe, with around 70% of them belonging to Cicadellidae. Since many species in this family are pests or vectors of plant pathogens, these introductions could have negative impacts on agriculture. Furthermore, it is important to conduct more faunistic studies to determine the natural ranges of species, which will help to identify when a non-native species is introduced.

This work is the result of monitoring activities carried out by the authors as part of biodiversity studies and *X. fastidiosa* vector species monitoring in several localities in Western Andalusia. These efforts have resulted in the collection of numerous Cicadellidae specimens, including several new or significant faunistic records of nonnative species. An overview of these records is provided together with their implications, taking into account the agronomic importance of the discovered species.

# MATERIAL AND METHODS

## Study area

The data were collected in the southwest of Andalusia (Spain), specifically within the Guadalquivir depression, through the provinces of Córdoba, Cádiz, Sevilla, and Huelva. This region covers a total area of 17,500 km<sup>2</sup>, with approximately 75% of it used for agriculture. The climate is typical Mediterranean, with an average annual temperature ranging from 18–19 °C. The region also has an average annual precipitation of 500–700 mm, and benefits from a high level of sunshine, with annual sunshine hours exceeding 3.000 (Land Use Information System of Spain (SIOSE 2020) and Climatic Regions of Andalusia; Andalusian Environmental Information Network (REDIAM); Fig 1).

# **Insect sampling**

The specimens were collected during regular surveys targeting *X. fastidiosa* vectors, and biodiversity of arthropods in berry crops. We used an entomological field aspirator (InsectaZooka®, Bioquip Products, Rancho Dominguez, California, USA) for this purpose. Monthly, from 2019 to 2022, five-minute aspirations were carried out on spontaneous vegetation located both in the perimeter and interior of agricultural plots, at 14 different locations (Table 1). The captured insects were transferred to Ziploc® bags. These bags were then placed in a cooler containing freeze packs for transportation to the laboratory. In the laboratory, the collected insects were preserved in a freezer at -20 °C until they could be identified.



FIGURE 1. Map with species records by localities in Western Andalusia. Representative images of sampling areas.

List of the localities, their coordinates and codes								
Province	Locality name	Labels map (Fig 1)	Coordinates	Altitude (mas.l.)	Plot description			
Cádiz	Rancho Cortesano	Cal	36°39'28" N 6°00'26" W	57	Vegetation cover of woody (almond, olive and vineyard) and herbaceous (potatoes) plots.			
Córdoba	Campus Rabanales 1	Col	37°55'06" N 4°43'17" W	142	Vegetation cover within olive plot. There is an artificial water channel a few meters away.			
	Campus Rabanales 2	Co2	37°55'10" N 4°43'22" W	142	Vegetation cover within Citrus and vineyard plot. There is an artificial water channel a few meters away.			
Huelva	Cañada del Corcho	Hu1	37°15'25" N 7°17'46" W	45	Spontaneous vegetation surrounding small fruits (strawberries, blueberries) and woody crop (sweet orange) plots. The samplings were carried out at the edge of a seasonal stream.			
	Arroyo de Vascogil	Hu2	37°17'01" N 7°14'36" W	42	Spontaneous vegetation near strawberry and Citrus plots.			
	San Bartolomé de la Torre	Hu3	37°25'49" N 7°04'48" W	104	Road verges vegetation and fallow land near to olive plots.			
	San Isidro	Hu4	37°25'17" N 7°02'32" W	97	Vegetation cover of pine forest stand (Pinus pinea L.)			
	Palos de la Frontera	Hu5	37°0'40.6" N 6°51'51.1" W	27	Ruderal vegetation adjacent to strawberry field drainage channel			
	El Cebollar	Hu6	37°14'24" N 6°48'9" W	65	Vegetation cover of pine forest stand (Pinus pinea L.)			
	Camino del Chaparral	Hu7	37°16'23" N 6°35'17" W	84	Vegetation cover of pine forest stand (Pinus pinea L.)			
	El Rocío	Hu8	37°08'48" N 6°29'37" W	13	Pine forest with remnants of Mediterranean scrub and ruderal vegetation adjacent to strawberry plots. The samplings were carried out at the edge of a seasonal stream.			
Sevilla	Alcalá del Río	Sel	37°30'52" N 5°58'03" W	11	Herbaceous vegetation within Citrus plot.			
	Cantillana	Se2	37°38'05" N 5°48'34" W	106	Ditch vegetation, near to olive plots.			
	Cantillana	Se3	37°38'14" N 5°48'32" W	96	Ditch vegetation, near to olive plots.			

<b>TABLE 1.</b> Description of the sampling locations,	their coordinates, and the	ir labels on the map in Figure 1
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# Identification

External morphological and genital structures were used for species identification. For genitalia examination, we dissected the abdomen of males and boiled it for a few minutes in a 10% KOH solution, followed by several times rinses in distilled water and then mounted in glycerol. As females generally lack reliable characters for identification, they were assigned by comparison with male specimens collected in the same samples. Genital structures were observed under a stereomicroscope (NIKON® SMZ 1500) and later stored in a microvials with glycerine, pinned below each specimen or glued with Dimethyl Hydantoin-Formaldehyde (DMHF) resin on small cards beneath the specimens. Illustrations of the genital structures were made using a drawing camera attached to a microscope (Olympus® BX50), and the final plates were prepared using Adobe Photoshop CS 8.0.

Species in this study were identified according to several key sources. *Balclutha incisa* (Matsumura) was identified according to Knight (1987, p. 1207 and figs. 138–145), and Khatri *et al.* (2011, at p. 749 and fig. 2).

*Cicadulina (Cicadulina) bipunctata* (Melichar) was based on Webb (1987, p. 600 and figs. 70–77), and Khatri *et al.* (2011,t p. 748 and fig. 1. For *Maiestas angustisecta* (Linnavouri) was used Webb & Viraktamath (2009, p. 58 and fig. 42), and the original description as *Deltocephalus angustisectus* by Linnavuori (1962, pp. 55–56, fig. 34.b).

Vouchers of the specimens studied have been deposited in the following collections: Collection of D. Aguín-Pombo, UMa Madeira, Portugal (DAPC), Insect Collection of the University of Madeira, Madeira, Portugal (UMACI), and Entomological collection of IFAPA, Las Torres, Sevilla.

## RESULTS

Below we provide descriptions of the physical characteristics of three newly discovered leafhopper species: *Balclutha incisa, Cicadulina bipunctata*, and *Maiestas angustisecta*. For each species, information is provided about their distribution in Andalusia, and potentially in other European regions. Additionally, details are provided about habitat preferences, the types of plants they are associated with and the potential role as vectors of plant pathogens.

#### Auchenorrhyncha Dumeril

**Cicadomorpha Evans** 

Cicadellidae Latreille

## **Deltocephalinae Fieber**

## **Tribe Macrostelini Kirkaldy**

The tribe Macrostelini presently comprises 37 genera, including *Balclutha* and *Cicadulina. Balclutha* is a cosmopolitan genus with approximately 120 species primarily associated with grasslands, exhibiting relatively uniform external morphology. The majority of its species are found in the New World, Africa and the Oriental Region (Knight 1987).

# Balclutha incisa (Matsumura)

(Figs 2-5A)

Gnathodus incisus Matsumura Balclutha breviceps Matsumura Balclutha ogasawarensis Matsumura Balclutha akonis Matsumura Eugnathodus bisinuatus DeLong Eugnathodus flavidus Naudé Eugnathodus pallidus Osborn Eugnathodus indica Singh-Pruthi Eugnathodus bifurcata DeLong & Davidson Nesosteles tutuilana Osborn Nesosteles areolata Osborn Eugnathodus juventus Van Duzee Empoasca athertoni Evans Cicadulina uniformis Metcalf Balclutha hortensis Lindberg Balclutha virescens Haupt Balclutha modesta Ahmed, Murtaza & Malik



FIGURE 2. Male adult of *Balclutha incisa* (Matsumura). (A) Head, pronotum and scutellum of male, dorsal view. (B–M) Male genitalia: (B) Pygofer, left lateral view; (C) Pygofer, posterior; (D) Pygofer process, ventral view; (E–F) Subgenital plate, lateral and ventral view, respectively; (G) Genital valve, dorsal view; (H) Connective, ventral view; (I) Style, left dorsal view; (J–M) Aedeagus: ventral, dorsal, lateral view. Due to damage during handling of the genitalia, the structures were drawn from a specimen from Cape Verde, collection code CV. 2010 085. Except for H and M, which belong to the specimen from Gibraleón, Huelva (Spain), 18 September 2019. Scale: 0.5 mm for A and 0.1 mm for B–M.

## Material examined

SPAIN. ANDALUSIA. **Huelva**: • 1  $\Diamond$ ; Gibraleón, 'San Isidro'; 97 m.a.s.l; 37°25'17"N, 7°02'32"W; 18.IX.2019; J.M. Molina leg.; on herbaceous vegetation near to berries crops. • 2  $\Diamond$ , 3  $\heartsuit$ ; ibid.; 13.XII.2021; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 8.II.2022; idem. leg.; idem. • 1  $\heartsuit$ ; Moguer, 'El Cebollar'; 65 m.a.s.l; 37°14'24"N, 6°48'9"W; 11.I.2022; L. Avivar leg.; on herbaceous vegetation near to berries crops. • 1  $\Diamond$ , 3  $\heartsuit$ ; Rociana del Condado, 'Camino del Chaparral'; 84 m.a.s.l; 37°16'23"N, 6°35'17"W; 13.XII.2021; J.M. Molina leg.; on herbaceous of open-scrub near to vineyard. • 3  $\heartsuit$ ; San Bartolomé de la Torre; 104 m.a.s.l; 37°25'49"N, 7°04'48"W; 16.XI.2021; L. Avivar leg.; on herbaceous vegetation near to olive crops. • 1  $\heartsuit$ ; ibid.; 11.I.2022; idem leg.; idem. Sevilla: • 2  $\Diamond$ , 2  $\heartsuit$ ; Alcalá del Río; 11 m.a.s.l; 37°30'52"N, 5°58'03"W; 30.IX.2020; J.M. Molina leg.; on herbaceous vegetation near to citrus crops. • 1  $\Diamond$ , 1  $\heartsuit$ ; Cantillana; 96 m.a.s.l; 37°38'14"N, 5°48'32"W; 29.XI.2019; L. Avivar leg.; on herbaceous vegetation near to olive crops. • 1  $\heartsuit$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. leg.; on herbaceous vegetation near to citrus crops. • 1  $\Diamond$ , 1  $\heartsuit$ ; ibid.; 12.XII.2019; idem. leg.; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.II.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 12.XII.2019; idem. leg.; idem.

# Description

These leafhoppers are small and elongate, with pale greenish colouration and head roundish featuring a semicircular vertex in dorsal view (Fig 5A).

**Head.** Ocelli whitish, antenna and eyes pale cream. Vertex greenish yellow (Fig 2A). Frontoclypeus yellowish. Head rounded in dorsal view, slightly broader than pronotum; anterior margin semicircular, 4x broader than long; width of vertex uniform; ocelli large on anterior margin of vertex.

**Thorax.** Pronotum, scutellum, prosternum, mesosternum and metasternum pale yellow. Pronotum 2.3x wider than longer; anterior margin concave, posterior margin slightly arcuate, with chamfered posterior margins. Wings 1.4 x longer than abdomen. Forewings hyaline, pale green, apical cells fumose, veins whitish. Legs greenish with dark cream claws.

**Abdomen.** Dorsal half dark brown, remaining parts pale-yellow green. In females, 7<sup>th</sup> sternite 2.5x wider than long, posterior margin slightly incised medially, lobe heavily sclerotized with brown w-shaped mark, edges sinuous ending in a spine-like projection not reaching the distal margin; 8<sup>th</sup> tergite extended to midlength of pygofer lobe.

**Male Genitalia.** Male pygofer elongate, rounded caudally, with a row of 8–9 macrosetae along distal third (Figs 2B–C); pygofer process brown at posteroventral margin, remaining parts yellow green, margins entirely or irregularly dentate (Fig 2C). Subgenital plates strongly bent outwards/ dorsally, basally subtriangular, narrower at its distal end, with 5 macrosetae along ventrolateral margin and long, thinner apical setae. Genital valve sub equilateral in ventral view (Fig 2G). Connective Y-shaped, expanded arms slightly bifid; stem 2x arm's length (Fig 2H). Style wide, apically with developed prominent hook-like apophysis curved laterally (Figs 2F–E), distal margin of lobe triangular with a tuff of microsetae. In the lateral view, aedeagus shaft slender, strongly arcuate, distally very thin and tubular, middle half 5x thinner than at base, apex with strong incision; aedeagus base triangular in dorsal view, with pair of thin long processes extended upwards (Figs 2J–M); apodemes greatly expanded with three pairs of processes extending posteriorly from base.

**Remarks.** For most species of this genus, reliable identification is based on the shape of the male genitalia (Knight 1987). The aedeagus of *B. incisa* typically has two or three pairs of processes. In lateral view, the aedeagus of the studied specimens appears distally wider compared to the illustrations of Lindberg (1958), and the connective stem is thinner. In females, the posterior margin of the 7th sternite displays a more pronounced sinuous shape, with roundish outer margins, in contrast to the almost concave shape depicted in Lindberg's (1958) illustrations. Records from Spain include *Balclutha frontalis* (Ferrari), *Balclutha punctata* (Fabricius) and *Balclutha saltuella* (Kirschbaum) (Bolívar & Chicote 1878; Nast 1972; Llacer *et al.* 1986).

## Distribution

This species has been sporadically documented in subtropical areas across all continents, ranging from central United States to Paraguay, spanning the Australian region, Africa, and South Asia, including countries from India to Japan and Russia. There are also reports of its presence on the Madeira Islands. Knight (1987), in his comprehensive genus revision, mentioned the presence of this species in the Canary Islands. This species has previously been reported in several Mediterranean countries. However, this marks the first recorded instance of its presence on the European mainland. Previous records from the Mediterranean region include Palestine and the island of Cyprus. In Cyprus, it was initially described as *B. hortensis* by Lindberg (1948, p. 140) but later synonymised to *B. incisa* by Knight (1987, p. 1207). Haupt (1927, p. 37) also described this species from Palestine as *B. virescens*, but it was subsequently synonymised by Linnavuori (1975, p. 631). In Andalusia (Spain), the first detection occurred in September 2019 in samples collected in Gibraleón, Huelva.

## Habitat and food plants

This insect primarily feeds on grasses (Narhardiyati & Bailey 2005). Knight (1987) has documented its foodplant preferences, listing the following plants: *Cynodon dactylon, Eriochloa subglabra, Oryza sativa, Brachiaria mutica* and *Saccharum* sp. (Poaceae), *Cyperus odoratus* (Cyperaceae), *Daucus carota* (Umbelliferae) and *Ipomoea batatas* (Convolvulaceae). In Egypt, *Balclutha incisa* was among the top three dominant species found in vegetable crops like zucchini, eggplant, maize, and rice (El-Wakeil *et al.* 2015).

In Spain, it has been found in two provinces: Huelva and Sevilla. In Huelva, it has been found in the natural herbaceous vegetation of pine forest stands (Fig 1, labels map: Hu4, Hu6, and Hu7; Table 1.) and along road verges (Fig 1, label map: Hu3). In Sevilla, it has been associated with citrus and almond crops on an experimental farm and has also been recorded in adventitious vegetation along the edges of a roadside ditch (Fig 1, labels map: Se1 and Se3, respectively; Table 1).

## **Tribe Macrostelini Kirkaldy**

Within the genus *Cicadulina*, there are two subgenera, namely *Idyia* and *Cicadulina* s. str., The subgenus *Idyia* is represented by a single species. In contrast, the subgenus *Cicadulina* s. str. is more diverse, comprising 22 recognizable species.

*Cicadulina (Cicadulina) bipunctata* (Melichar) (Figs 3–5B)

Gnathodus bipunctatus Melichar Cicadula bipunctella Matsumura Cicadulina zeae China Cicadulina bipunctella bipunctella (Matsumura) Cicadulina bipunctella zeae China Cicadulina bipunctata (Melichar)



FIGURE 3. Adult of *Cicadulina (Cicadulina) bipunctata* (Melichar). (A) Female forewing, right dorsal view; (B) Head, pronotum and scutellum of male, dorsal view. (C–L) Male genitalia: (C) Pygofer, left lateral dorsal view; (D) Pygofer process, dorsal view (i–ii right and iii–v left); (E) Subgenital plate, dorsal view; (F) Genital valve, dorsal view; (G–J) Aedeagus: lateral (G–H), ventral (I) and dorsal (J) views; (K) Connective, ventral view; (I) Style, right dorsal view. Specimens from Huelva and Sevilla (Spain), 15 September 2020, 13 December 2021, 13 January 2022 and 07 February 2022. Scale: 0.5 mm for A–B and 0.1 mm for C–L.

#### Material examined

SPAIN. ANDALUSIA. Cádiz: • 1  $\bigcirc$  1  $\bigcirc$ ; Jérez de la Frontera, 'Rancho Cortesano'; 57 m.a.s.l; 36°39'28"N, 6°00'26"W; 14.XII.2021; L. Avivar leg.; on herbaceous vegetation near to vineyard.

**Córdoba**: • 1  $\bigcirc$ ; Córdoba 'Campus Rabanales 1'; 142 m.a.s.l; 37°55'06''N, 4°43'17''W; 24.X1.2021; L. Avivar leg.; on herbaceous vegetation near to olive. • 1  $\bigcirc$ ; 'Campus Rabanales 2'; 142 m.a.s.l; 37°55'10''N, 4°43'22''W; 22.X.2019; S. Pérez leg.; idem.

**Huelva**: • 1  $\Diamond$ , 1  $\heartsuit$ ; Almonte, 'El Rocio'; 13 m.a.s.l; 37°08'48"N, 6°29'37"W; 11.XI.2020; J.M. Molina leg.; on herbaceous vegetation near to strawberries crops. • 3  $\heartsuit$ ; Rociana del Condado, 'Camino del Chaparral'; 84 m.a.s.l; 37°16'23"N, 6°35'17"W; 30.XI.2021; J.M. Molina leg.; on herbaceous of open-scrub near to vineyard. • 1  $\Diamond$ , 1  $\heartsuit$ ; ibid.; 13.XII.2021; idem. leg.; idem. • 1  $\Diamond$ , 1  $\heartsuit$ ; 13.I.2022; idem. leg.; idem. • 1  $\Diamond$ , 1  $\heartsuit$ ; Lepe, 'Arroyo de Vascogil'; 42 m.a.s.l; 37°17'01"N, 7°14'36"W; 19.XI.2020; S. Pérez leg.; on herbaceous vegetation near to strawberries crops. • 4  $\Diamond$ , 2  $\heartsuit$ ; 'Cañada del Corcho'; 45 m.a.s.l; 37°15'25"N, 7°17'46"W; 19.XI.2020; L. Avivar leg.; on herbaceous vegetation near to berries crops.

Sevilla: • 1  $\Diamond$ , 1  $\Diamond$ ; Alcalá del Río; 11 m.a.s.l; 37°30'52"N, 5°58'03"W; 27.VIII.2020; J.M. Molina leg.; on herbaceous vegetation near to citrus crops. • 1  $\Diamond$ , 2  $\Diamond$ ; ibid.; 15.IX.2020; idem. leg.; idem. • 1  $\Diamond$ , 1  $\Diamond$ ; ibid.; 19.X.2020; idem. leg.; idem. • 1  $\Diamond$ ; Cantillana; 106 m.a.s.l; 37°38'05"N, 5°48'34"W; 18.XI.2020; L. Avivar leg.; on herbaceous vegetation near to olive crops. • 1  $\Diamond$ ; 96 m.a.s.l; 37°38'14"N, 5°48'32"W; 30.I.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 30.X.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 18.XI.2020; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 15.V.2021; idem. leg.; idem. • 1  $\Diamond$ , 1  $\Diamond$ ; ibid.; 10.XII.2021; idem. leg.; idem. • 1  $\Diamond$ , 1  $\Diamond$ ; ibid.; 07.II.2022; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 26.IV.2022; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 27.V.2022; idem. leg.; idem. (Fig 1, Ca1, Co1, Co2, Hu8, Hu7, Hu1, Hu2, Se1, Se2, Se3).

#### Description

Small yellow-orange leafhoppers, easily distinguished by two round black spots on the vertex (Fig 5B).

**Head.** Ocelli, antenna, and eyes pale yellow. Vertex yellow orange with two large oval black spots on anterior margin (Fig 3B). Frontoclypeus yellowish. Head in dorsal view rounded, broader than pronotum; vertex produced, exceeding the anterior margin of the eyes and longer medially than half the width between the eyes (Fig 3B). Inner margin of eyes often whitish, posterior half dark brown to black. Ocelli on anterior margin of vertex, below black spots.

**Thorax.** Pronotum golden orange, paler behind the eyes and at posterior margin; 2x as long as vertex and semicircular in shape with posteriorly divergent lateral margins (Fig 3B). Scutellum yellowish, paler in the middle at posterior margin. Prosternum, mesosternum and part of metasternum yellowish. Wings subhyaline and longer than abdomen. Forewings orangish anteriorly but fumose towards the apex, pale yellow veins, with three apical cells. Hindwings with dark brown veins. Legs pale yellow with dark brown tarsal claws; hind tibiae externally with a row of stout setae, sometimes with brown base.

**Abdomen.** Basal dorsal half of male abdomen suffused with dark brown, last two sternites with a light brown trip medially. Abdomen of females almost entirely black with some light tinges of yellow along the lateral margins; 7<sup>th</sup> sternite yellowish with some brown marking in the middle, 3x wider than 6<sup>th</sup> sternite, and outer margins at posterior margin rounded. Medially, with a strongly produced sinuous edges ending in a spine-like projection without reaching distal margin. Pygofer dorsally yellow-brown, while distally and around macrosetae basis, pale yellow. In females, distal margin of 8<sup>th</sup> tergite extends about two-thirds of pygofer lobe. Ovipositor yellowish heavily pigmented distally.

**Male Genitalia.** Pygofer pale yellow with a dark brown stripe, inner margins along subgenital plate dark brown; deeply incised dorsally, in lateral view triangular with 7–8 macrosetae dorsally; caudally, with a long, slender hook-like process with smooth margins (Fig 3C). Subgenital plate narrow apically, ending in a finger-shape elongation that vents outward; 4 macrosetae medially along ventrolateral margin (Fig 3E). Genital valve triangular in ventral view (Fig 3F). Connective Y-shaped, arms slightly shorter than stem (Fig 3K). Style apex hooked with a strongly bent process, and apical margin triangular with a small tuff of thin setae (Fig 3L). In lateral view, aedeagus shaft cylindric, strongly arcuate with a blunt distal part, slightly wider at its apical half; basis of shaft ventrally quadrangular with a pair of spine-like processes, gonopore apical (Figs 3G–J).

**Remarks/diagnoses.** This species is primarily distinguished by the hook-like apex of the pygofer processes. However, there is considerable intraspecific variation in male genitalia. Main diagnostic characters include 2–3 spines of subequal or different size on the ventral margin of the aedeagus and 1–2 spines on the pygofer process. There is intraspecific variation in lateral spines; they may be absent or present up to three in the number, and their position may vary. All specimens studied have two spines equal or subequal in size in the aedeagus, but the pygofer process exhibits some variation. *C. bipunctata* is the most widespread and variable species of the genus (Webb 1987) and closely resembles *Cicadulina bimaculata* (Evans). The main distinguishing features are that *C. bimaculata* has 2–5 subapical spines on the pygofer and the aedeagal shaft with 2–5 dorsal processes, whereas *C. bipunctata* has 1– 3 subapical spines on the pygofer and 0–3 dorsal aedeagal processes. Those characters are partially overlapping, and Webb (1987) also included distributional information in the key to separate these similar species, with *C. bipunctata* being more widespread, known from the southern Palaearctic, Old World tropics, N. Australia, and Pacific and *C. bimaculata* known from Australia (Queensland, New South Wales), New Caledonia, and Vanuatu.

## Distribution

*C. bipunctata* is recorded from Africa, Asia, Australia, and South America. In the Palearctic, it is widespread in north African Mediterranean countries, extending from Tunisia to Turkey, which encompasses Cyprus, Lebanon, the island Thira (Greece) and the Macaronesia regions (Madeira Islands and the Canary Islands). Notably, it has not been reported from Morocco. This study marks the first recorded occurrence of *C. bipunctata* in southern Europe.

The first detection in Andalusia took place in October 2019 in samples collected in Rabanales (Córdoba). Subsequently, it was detected Huelva and Sevilla provinces. Additional records were obtained by tracking photo-sharing websites such as GBIF (https://www.gbif.org/es/), Biodiversidad Virtual (http://www.biodiversidadvirtual. org/); iNaturalist (https://www.inaturalist.org/) and Observation.org (https://observation.org/). These online platforms show a broader distribution in the southern parts of the Iberian Peninsula, including Alicante (August 2012), Almeria (November 2022, August 2023), Granada (March 2015), Murcia (August 2012 and November 2011), and Valencia (January 2020 and August 2018). There are also records from Portugal (Silves in Portimão) in September 2021 and from Crete (Greece) in August 2022 and September 2012. This information suggests that certain southern parts of Europe may represent the northern limit of its distribution range.

## Habitat and food plants

The reports of habitat preferences and plant associations of *C. bipunctata* differ among regions. In the Canary Islands, it was collected on plants belonging to the Chenopodiaceae family and *Mesembryanthemum* species. These findings were primarily observed in plains, on the edges of cultivated areas (Carl 1995; Lindberg 1953).

In Andalusia, this species has been found in ten distinct locations, each characterised by specific habitat types and vegetation association (Fig 1, Table 1). In the locations Córdoba ('Rabanales 1–2'), Huelva ('Cañada del Corcho', 'El Rocio') and Sevilla ('Cantillana'), it was found in spontaneous vegetation, often occurring in the proximity of intermittent natural or artificial watercourses. In Cadiz ('Rancho Cortesano'), Huelva ('Camino del Chaparral'), and Sevilla ('Alcalá del Río'), it was found in herbaceous vegetation that is typically associated with cultivated crops. These observations highlight the species' adaptability to a range of habitat types and its ability to inhabit both natural and cultivated environments, depending on the location.

## **Tribe Deltocephalini Dallas**

This tribe comprises 76 genera and an estimated 598 species. The genus *Maiestas* Distant, initially established by Distant in 1917 includes the type species *Maiestas illustris* Distant. The genus *Maiestas* is particularly large with 112 species (Webb & Viraktamath 2009; Zhang & Duan 2011; Dmitriev *et al* 2022).

# Maiestas angustisecta (Linnavuori)

(Figs 4-5C)

Deltocephalus angustisectus Linnavuori Recilia dolabra Kramer Recilia jordanica Dlabola



FIGURE 4. Male adult of *Maiestas angustisecta* (Linnavuori). (A) Head, pronotum and scutellum of male, dorsal view; (B) Head of male, ventral view. (C–J) Male genitalia: (C) Subgenital plate and genital valve, ventral view; (D) Pygofer, left lateral view; (F) Right style, dorsal view; (E) Connective, dorsal view; (G–J) Aedeagus, dorsal (G–H) and left lateral (I–J) view. Scale: 0.5 mm for A–B and 0.1 mm for C–J. (A–F and H–J) Specimens from Madeira Island and (G) Specimen from San Bartolomé de la Torre, Huelva, (Spain),11 January 2021.



**FIGURE 5.** Habitus of three species. (A) Female adult of *Balclutha incisa* (Matsumura). (B) Female adult of *Cicadulina* (*Cicadulina*) bipunctata (Melichar). Male adult of (C) Maiestas angustisecta (Linnavuori).

## Material examined

SPAIN. ANDALUSIA. **Huelva**: • 1  $\Diamond$ ; Palos de la Frontera; 27 m.a.s.l; 37°10'40.6"N, 6°51'51.1"W; 8.X.2020; L. Avivar leg.; on herbaceous vegetation near to strawberries crops. • 1  $\Diamond$ ; San Bartolomé de la Torre; 104 m.a.s.l; 37°25'49"N, 7°04'48"W; 29.IV.2021; idem. leg.; on herbaceous vegetation near to olive crops. • 1  $\Diamond$ ; 1  $\Diamond$ ; ibid.; 18.XII.2021; idem. leg.; idem. • 1  $\Diamond$ ; ibid.; 11.I.2022; idem. leg.; idem.

Sevilla: • 1 ♂; Cantillana; 96 m.a.s.l; 37°38'14"N, 5°48'32"W; 7.II.2022; idem. leg.; idem. (Fig 1, Hu5, Hu3, Se3)

## Description

Medium-sized leafhoppers, characterized by their straw pale colouration and distinctive features, including a triangular vertex and head larger than pronotum (Fig 5C).

**Head.** Head in dorsal view distinctively triangular, broader than pronotum; vertex pentagonal with slightly rounded tip, 1.2x broader than long. Approximately half of vertex extended anterad of eyes. The forehead yellowish with a variable number of small, transverse brown lines (Fig 4B). Small ocelli on anterior margin, close to eyes.

**Thorax.** Pronotum brownish with a distinctive brown U-shaped longitudinal stripe (Fig 4A).Scutellum yellowish, with two triangular, slightly brownish spots at anterior margin (Fig 4A); approximately 1.5x wider at basis than medially, narrowing distinctly towards its distal end. Wings longer than the abdomen, can be pale straw or with distinct dark markings. Hindwings whitish with fumose veins.

**Abdomen.** The abdomen primarily brown with few yellowish spots ventrally. Brown transverse bands mark on abdominal segments, not visible through the wings. The junction lines between abdominal segments yellowish, but not particularly conspicuous.

**Male Genitalia.** Male pygofer elongate, deeply incised dorsally, and rounded caudally, with a row of 8–9 macrosetae along the distal third (Fig 4D); margins at postero-central side either entirely or irregularly dentate. Pygofer triangular and on the posterior caudo-dorsal side with a mixture of short and long setae along the outer edge. Subgenital plate like a circular sector, as wide as long with arch-shaped outer margin bearing 8 long macrosetae. On the caudo-ventral side, a row of long setae along the outer margin (Fig 4C). In ventral view, genital valve subequilateral with a M-shaped projection at the tip (Fig 4C). Connective Y-shaped, arms expanded and slightly bifid with stem twice the length of as arms (Fig 4E). Style fish-tail shaped, wider basally than apically and with a prominent hook-like apophysis at apex, which is bent laterally (Fig 4F). Distal margin of style lobe triangular with a tuff of microsetae. In lateral view, aedeagus shaft tubular, curved, distal third with a ventral projection twice as wide (Figs 4I–J). In dorsal view, aedeagus basis triangular, shaft slender and tubular slightly thinner in the middle, curved anteriorly; apex pear-shaped covered at its distal third with a veil-like expansion facing upwards; veil-like structure twice wider at the base, with serrate inner margins and gonopore distal (Fig 4H).

**Remarks.** The aedeagus of this species is like that of *Maiestas coronata* (Melichar) and *Maiestas lobata* (Linnavuori). It was described from Israel and Palestine by Linnavuori in 1962.

#### Distribution

This species has scattered records from various parts of Africa, including Liberia, Congo, Cote d'Ivoire and Democratic Republic of Congo (DRC). It is worth noting that the record from DRC requires confirmation, as indicated by Webb & Viraktamath 2009. This species extends its range into the eastern Mediterranean: Israel, Palestine, Lebanon, Jordan, and Syria. These findings suggest that the species may have a broad distribution across the Mediterranean basin, with the northern boundary of its range extending into south of Europe the northern limit of its distribution range.

In Andalusia, it has been found in two provinces, Huelva and Sevilla, specifically in three locations labelled as Hu3, Hu5, and Se3 (see Figure 1, labels map).

## Habitat and food plants

Very little information is available about the biology and habitat preferences of this species. In Andalusia, specimens were collected at the edges of cultivated fields where ruderal vegetation was prevalent. However, in 'Palos de la Frontera' and 'Cantillana' (Table 1), the boundaries of the collection sites coincided with channels for the collection of excess irrigation and rainwater. In 'San Bartolomé de la Torre', the sampling was conducted in road verges. The surrounding vegetation included various plant species, with grasses such as *Avena barbata*, *C. dactylon* and *Sorghum halepense* (Poaceae).

## DISCUSION

The subfamily Deltocephalinae within the Cicadellidae family includes approximately 60% of vector species responsible for transmitting 70% of known phytopathogenic agents (Dakhil *et al.* 2011). For instance, the orange leafhopper *C. bipunctata* serves as a vector for the transmission of maize wallaby ear symptom (MWES) (Grylls 1975) and maize streak disease (Rose 1978). More recently, it has been found that *C. bipunctata* and *B. incisa* host *Candidatus Phytoplasma asteris*, 16SrI-B, and *Candidatus Phytoplasma solani*, 16SrXII-A, respectively (Abu Alloush *et al.* 2023). Plants infected with *Ca. P. asteris* can exhibit symptoms such as leaf yellowing, growth

abnormalities, including malformed flowers and fruits, as well as stunted growth. The symptoms associated with *Ca. P. solani* depend on environmental factors and are associated to diseases like bois noir and stolbur. Stolbur is classified as a quarantine pest in the EPPO A2 list (EPPO, 2023). Both phytoplasmas can affect a wide range of wild and cultivated herbaceous crops and woody plants (Lee *et al.* 2004; Pedraza *et al.* 2022).

*B. incisa* has been reported as a putative vector of '*Ca. P. australasia*' (16SrII-D), which is associated with fenugreek phyllody in Pakistan (Malik *et al.* 2020). It has also been associated with 16SrII phytoplasmas responsible for cactus witches-broom in Indonesia and yellow disease in carrots (Wulandari *et al.* 2021).

There are no references supporting the role of *M. angustisecta* as a vector of any phytopathogen. However, it is important to note that several species within the *Maiestas* genus are known to be important vectors of phytoplasmas (Mathur & Chaturvedi 1980; Ramya *et al.* 2017). For example, *Maiestas portica* (Melichar) has been reported as a vector of sugarcane grassy shoot disease (SCGS) associated with phytoplasmas of the 16SrXI group in India (Tiwari *et al.* 2017). Similarly, *Maiestas dorsalis* (Motschulsky) has been identified as a vector of rice orange leaf disease (ROLP) linked to phytoplasmas of the 16SrI group in Southeast Asia (Li *et al.* 2015 and references therein; Ong *et al.* 2021). Therefore, we cannot rule out its potential to act as a vector.

In our study area, the Guadalquivir Depression, agriculture plays a significant role, with 5,600 km<sup>2</sup> dedicated to tree crops, 500 km<sup>2</sup> to cereals, 352.3 km<sup>2</sup> to rice, and 23.5 km<sup>2</sup> to strawberries (Climatic Regions of Andalusia and SIOSE 2020 (REDIAM)). Many of these crops are susceptible to phytoplasma infections. The presence of these three potential vectors in this region raises concerns about the increased risk of phytoplasma-related diseases, which could pose a threat to the agricultural sector by potentially leading to production and trade losses. Furthermore, although these species do not directly feed on crops, such as woody crops, it is important to note that they can act as reservoirs for phytoplasmas, which can then be transmitted to other vectors.

The Auchenorrhyncha fauna in Andalusia is relatively understudied, presenting an opportunity for the discovery of new African species in the region. Considering the current distribution of the three species reported in this study, it is reasonable to suggest that the Iberian Peninsula represents the northern limit of their natural distribution range, rather than that they are introduced species that have gone unnoticed until now. Andalusia's strategic geographic location at the crossroads of Europe and Africa holds immense geological and biogeographical significance. This significance is particularly highlighted by its role as a corridor during the Messinian period, a geological era dating back approximately 7.2 to 5.3 million years in the Late Miocene.

The Messinian period was characterised by remarkable geological and climatic events, notably the Messinian Salinity Crisis (MSC), which led to the partial drying of the Mediterranean Sea. This event created a land bridge connecting the Iberian Peninsula to North Africa, facilitating the exchange of faunal species between Europe and Africa. This faunal interchange had a profound impact on the distribution patterns of biodiversity in the Mediterranean region.

Considering the limited knowledge of the Auchenorrhyncha fauna in Andalusia, there remains the possibility of discovering additional African species in the area. Such discoveries would further enhance our understanding of the biogeography and ecological history of this remarkable region. Additionally, climate change may promote the expansion of tropical or subtropical species from the south of the Mediterranean Basin and north Africa into southern Europe, altering the biodiversity of these ecosystems. This information strongly emphasizes the need for further research in the Iberian Peninsula, particularly in its southern region, which may play a crucial role in understanding the distribution of subtropical and tropical species.

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