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First record of *Amrasca* (*Sundapteryx*) *biguttula* (Ishida, 1913) (Hemiptera: Cicadellidae: Typhlocybinae) from Türkiye and its morphological and molecular identification

İBRAHİM ERSİN BOZDOĞAN[1], HAKAN ÇARPAR[1], NİHAT DEMİREL[1*] & ÖMER FARUK COŞKUN[2]
[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Dept. of Plant Protection, Hatay, Türkiye*
[2]*Hatay Mustafa Kemal University, Faculty of Agriculture, Dept. of Horticulture, Hatay, Türkiye*
ie.bozdogan@mku.edu.tr; https://orcid.org/0000-0002-9841-9744
hcarpar@mku.edu.tr; https://orcid.org/0000-0002-2511-4480
ndemirel@mku.edu.tr; https://orcid.org/0000-0002-3631-2458
omerfaruk.coskun@mku.edu.tr; https://orcid.org/0000-0001-5398-5737
**Corresponding author: ndemirel@mku.edu.tr*

Abstract

The two-spot cotton leafhopper *Amrasca biguttula* (Ishida, 1913) is a polyphagous cicadellid that has recently expanded from its native Asian range into Africa, the Caribbean and the southeastern United States. During faunistic surveys in 2024–2025, leafhoppers resembling *A. biguttula* were found on eggplant (*Solanum melongena* L.) and cotton (*Gossypium hirsutum* L.) in Hatay Province, eastern Mediterranean region of Türkiye. Adults and males were examined in detail, and both external characters and male genitalia matched the diagnostic features described for *A. biguttula*, including the two distinct preapical dark spots on the forewings and the characteristic shape of the aedeagus and subgenital plates. COI sequences generated from Hatay specimens were 99.4–100% identical to published sequences from India and Southeast Asia, and phylogenetic analyses grouped the Turkish haplotype within a well-supported *A. biguttula* clade. In infested cotton fields, both nymphs and adults caused typical hopperburn symptoms, such as marginal chlorosis, necrotic patches and leaf curling, indicating that the species is established and actively damaging local crops. Because cotton is a key industrial crop in southern Türkiye, early detection, routine monitoring and coordinated management efforts will be essential to limit the impact of this invasive leafhopper in the region. These findings provide the first morphologically and COI-confirmed record of *A. biguttula* from Türkiye and extend the westernmost known limit of the species into the eastern Mediterranean.

Key words: Two-spot cotton leafhopper, *Amrasca biguttula*, first record, invasive pest, COI barcode, phylogenetics, Türkiye

Introduction

The two-spot cotton leafhopper *Amrasca biguttula* (Ishida, 1913) (Hemiptera: Cicadellidae: Typhlocybinae) is a polyphagous leafhopper widely distributed in tropical and subtropical Asia. The species is a well-known pest of several solanaceous and malvaceous crops, including eggplant (*Solanum melongena* L.), cotton (*Gossypium hirsutum* L.), and okra (*Abelmoschus esculentus* (L.) Moench), where both nymphs and adults cause characteristic "hopperburn" symptoms due to feeding on leaf mesophyll cells. Severe infestations can lead to chlorosis, necrosis, leaf curling, and premature defoliation, resulting in substantial yield losses (Xu *et al.* 2017; Sagarbarria *et al.* 2020; Jaod & Nawar 2023; Azrag *et al.* 2025; Esquivel *et al.* 2025; Michel & Orozco 2025).

The taxonomy of *Amrasca* has long been confused because several synonymous names were used historically (e.g., *A. devastans* Distant, 1918). Xu *et al.* (2017) provided a comprehensive revision of the Chinese *Amrasca* species and designated a neotype for *A. biguttula*, stabilizing its identity and clarifying diagnostic morphological characters, particularly male genital and pregenital structures. Morphological identification of *A. biguttula* typically relies on the presence of two conspicuous black spots on the forewing and distinctive features of the male aedeagus,

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subgenital plates and pregenital abdominal tergites (Xu *et al.* 2017). Molecular identification using mitochondrial COI sequences has proven effective for confirming species identity and distinguishing *A. biguttula* from closely related taxa (Sagar *et al.* 2014; Akmal *et al.* 2017; Sagarbarria *et al.* 2020; Dung *et al.* 2021; Jaod & Nawar 2023).

In recent years, *A. biguttula* has expanded its range beyond Asia, being reported from West and Central Africa and the Caribbean Basin, and subsequently detected in Florida, USA (Liburd *et al.* 2024; Michel & Orozco 2025). Most recently, Michel & Orozco (2025) recorded the species for the first time in Central America (Honduras).

During field surveys conducted in Hatay Province (eastern Mediterranean region of Türkiye) in 2024–2025, specimens of *A. biguttula* were collected from eggplant and cotton fields. This paper provides the first confirmed record of *A. biguttula* from Türkiye, based on detailed external and male abdominal morphology supported by COI-based molecular data, and discusses its potential implications for cotton production in the eastern Mediterranean region. Diagnostic illustrations, field photographs, and a phylogenetic tree are presented to document this new occurrence.

Materials and methods

Sampling and morphological examination

Specimens of *Amrasca biguttula* were first collected in 2024 from eggplant (*Solanum melongena* L.) fields located in Antakya district, Hatay Province, eastern Mediterranean region of Türkiye, using a standard sweep net (45 cm diameter). Considering that *A. biguttula* is an important pest of cotton, additional field surveys were conducted in 2025 in cotton (*Gossypium hirsutum* L.) fields in Antakya and Reyhanlı districts. The species was detected from both host plants during these surveys (see Fig. 1).

All collected specimens were preserved at -18 °C until examination. Specimens collected in 2024 were first examined in early 2025. Under a stereomicroscope, individuals suspected to belong to *A. biguttula* were separated from other leafhoppers based on external morphology. Subsequently, males and females were sorted and preserved separately. Both sexes were examined under a stereomicroscope (Nikon SMZ1500) and photographed from dorsal, lateral, and ventral views using a digital camera (ToupTek XCAM-1080PHD) mounted on the microscope.

For detailed study of the male genitalia, the abdomen was carefully detached from the thoracic region with fine forceps and macerated in 10% potassium hydroxide (KOH) solution for one minute. The cleared abdomens were rinsed several times with distilled water, then transferred to depression slides containing glycerin. Male genital structures were dissected and positioned in glycerin under the stereomicroscope (Nikon SMZ1500, 40× magnification). Photographs of the genital capsule and associated parts were taken using the mounted camera system. All dissected genitalia were stored in microvials containing glycerin and pinned beneath the corresponding specimen.

The terminology used for morphological structures followed general Cicadellidae diagnostic standards (Borror *et al.* 1971) and specific descriptive characters provided for *A. biguttula* by Xu *et al.* (2017).

Data presentation and imaging

Photographs of specimens were taken by using a ToupTek XCAM-1080PHD digital camera mounted on a Nikon SMZ1500 stereomicroscope, operated through ToupView v.4.12 imaging software. Only minor adjustments of brightness and contrast were made when necessary, without altering any morphological features.

The distribution map was created by using ArcMap 10.8.2 software based on GPS coordinates recorded during field sampling. Sampling sites were marked according to host plants and detection status of *Amrasca biguttula* as indicated in Figure 1.

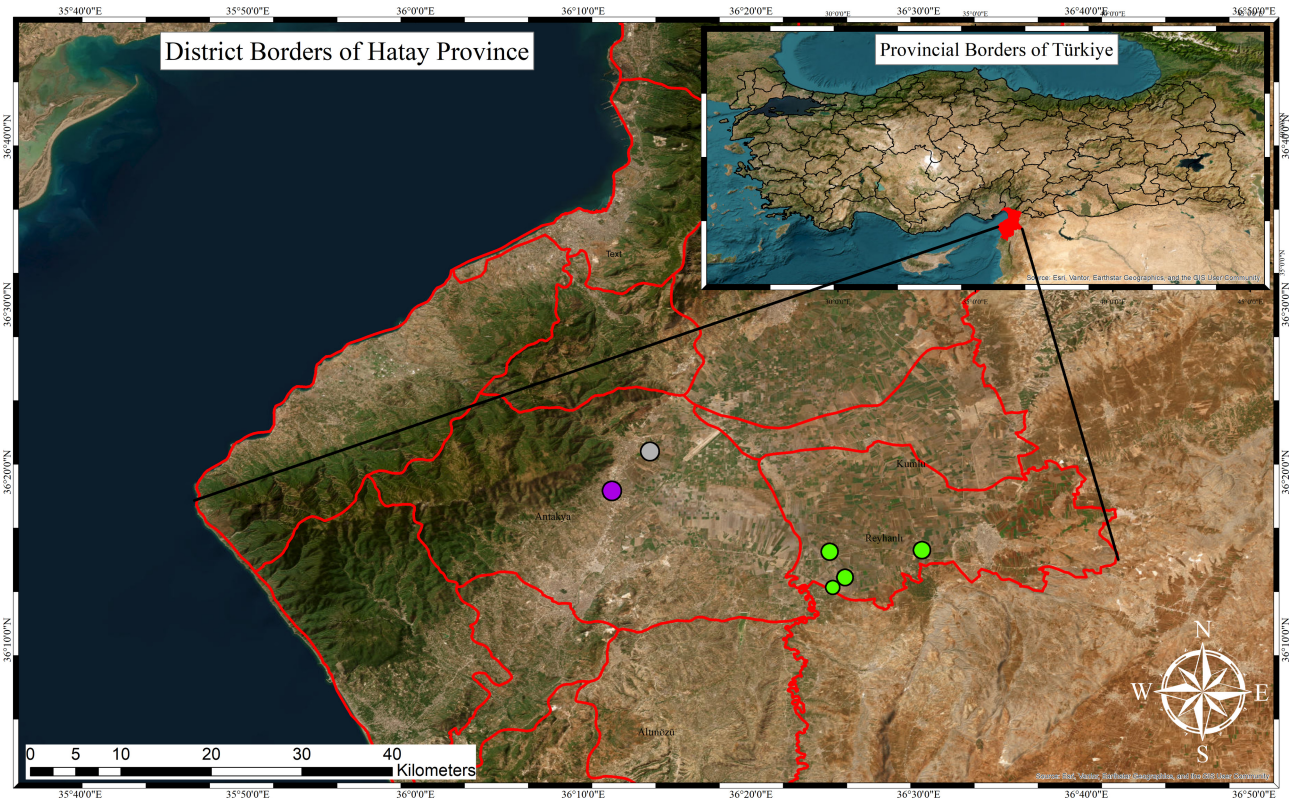

FIGURE 1. Sampling localities of *Amrasca biguttula* (Ishida, 1913) in Hatay Province, Türkiye. Green circles = cotton fields where *A. biguttula* was detected; purple circle = eggplant field where the species was detected; grey circle = sampling sites where *A. biguttula* was not detected. Red lines indicate district borders within Hatay Province; inset map shows the location of Hatay within Türkiye.

DNA extraction, COI amplification, and sequencing

Total genomic DNA was extracted from whole-body leafhopper samples using the NucleoSpin® DNA Insect Mini Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. The COI gene was amplified by using the universal primers LCO1490 (F) 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198 (R) 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer *et al.* 1994). The PCR reaction was carried out in a total volume of 25 µL containing 1× PCR buffer, $MgCl_2$ at the manufacturer-recommended concentration, 0.2 mM of each dNTP, 0.4 µM of each primer, 1 U of recombinant Taq DNA polymerase, and 1.5 µL of template DNA, with nuclease-free water added to volume. The PCR amplification was performed in a MiniAmp™ Plus Thermal Cycler under the following conditions: an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplified products were visualized by electrophoresis on a 1% TBE agarose gel. The gel was stained with RedSafe™ Nucleic Acid Staining Solution, and a 1 Kb plus DNA ladder (MassRuler DNA Ladders) was used as a molecular size marker. Gels were visualized under a UV transilluminator, and clear bands of the expected size (658 bp) were obtained for all successfully amplified specimens. The amplified COI gene fragments were sent to BMlabosis (Ankara, Türkiye) for purification and bidirectional Sanger sequencing using the same primers employed in PCR amplification.

COI sequence annotation and phylogenetic analyses

Sequences obtained from the isolates and reference sequences retrieved from GenBank were aligned by using MEGA X (v.10.2.4). Low-quality bases at both ends of the COI sequences were trimmed, and one cleaned isolate was

deposited in NCBI GenBank under the accession number PX453642. All sequences were aligned with the reference *A. biguttula* COI sequence (PP930924) using the MUSCLE algorithm and manually inspected. Sequence diversity was assessed based on the number of segregating sites, and node support in the phylogenetic tree was evaluated using 1,000 bootstrap replicates. Phylogenetic relationships were inferred by using newly generated COI sequences together with *A. biguttula* sequences and related taxa obtained from GenBank. Phylogenetic relationships were inferred by using both Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods, each with 1,000 bootstrap replicates, to evaluate the robustness of clustering patterns. Accession numbers, scientific names, localities, and references of all sequences included in the analysis are provided in Table 1. The species *Diaphorina citri* Kuwayama, 1907 was selected as the outgroup (Rwomushana *et al.* 2017).

TABLE 1. GenBank accession numbers, scientific names, collection localities, and references of *Amrasca biguttula* and related leafhopper COI sequences used in the phylogenetic analysis.

Accession number	Scientific name	Location	Reference
OR004178	*Amrasca biguttula*	India	Venkatesan & Venugopala 2023
MW363544	*Amrasca biguttula*	India	Ranebennur & Rao 2020
MN399898	*Amrasca biguttula*	India	Reddy *et al.* 2019
PP930924	*Amrasca biguttula*	India	Sridhar *et al.* 2024a
PP935748	*Amrasca biguttula*	India	Sridhar *et al.* 2024b
PX453642	*Amrasca biguttula*	Türkiye	This study
MW190078	*Amrasca splendens*	Vietnam	Dung *et al.* 2021
KY752061	*Cicadella viridis*	China	Han & Yu 2017
NC_024838	*Empoasca vitis*	China	Zhou *et al.* 2016
MG398807	*Empoasca fabae*	Canada	Dewaard 2017a
KR579805	*Hebata decipiens*	Canada	Hebert *et al.* 2016
KR044841	*Empoasca coccinea*	Canada	Gwiazdowski *et al.* 2015
MG404583	*Empoasca luda*	Canada	Dewaard 2017b
MT229895	*Diaphorina citri*	Kenya	Rwomushana *et al.* 2017

Results and discussion

Genus *Amrasca* Ghauri, 1967

Type species: ***Amrasca splendens*** Ghauri, 1967—by original designation.

Subgenus *Amrasca* (*Sundapteryx*) Dworakowska, 1970

Originally described as *Sundapteryx* Dworakowska (1970) based on *Chlorita biguttula* Ishida (1913); later treated as a junior synonym of *Amrasca* by Dworakowska & Viraktamath (1975).

This subgenus is characterized by macrosetae confined to the basal half of the subgenital plate and the presence of a pair of large lateral apodemes on pregenital tergite VII and arched internal ridges on tergite VIII.

Distribution. Oriental and Australian regions.

Amrasca (*Sundapteryx*) *biguttula* (Ishida, 1913)

Chlorita biguttula Ishida, 1913—type locality: Japan (Okinawa Islands).
Empoasca biguttula (Ishida): Shiraki, 1913; Dworakowska, 1970.
Zygina punctata Melichar, 1914 (syn.); *Chlorita bimaculata* Matsumura, 1916 (syn.); *Empoasca devastans* Distant, 1918 (syn.)—see Xu *et al.* (2017) for full synonymy.

Material examined. *Amrasca biguttula* (Ishida, 1913). Türkiye: Hatay province: Antakya district, Anayazı (36°18′52.0″ N, 36°11′54.3″ E; 104 m a.s.l.), on *Solanum melongena* (eggplant), sweep-net, 2024, coll. H. Çarpar (*A. biguttula* detected); same locality (36°18′52.0″ N, 36°11′54.3″ E; 104 m a.s.l.), on *S. melongena*, sweep-net, 4 Sep 2025, coll. I. E. Bozdoğan (*A. biguttula* detected).

Reyhanlı district: Tayfursökmen (36°13′03.9″ N, 36°25′24.0″ E; 89 m a.s.l.), on *Gossypium hirsutum* (cotton), sweep-net, 12 Sep 2025, coll. I. E. Bozdoğan (*A. biguttula* detected); Selam (36°12′56.5″ N, 36°25′06.5″ E; 89 m a.s.l.), on *G. hirsutum*, sweep-net, 12 Sep 2025, coll. I. E. Bozdoğan (*A. biguttula* not detected); Göktepe (36°15′18.7″ N, 36°30′13.2″ E; 95 m a.s.l.), on *G. hirsutum*, sweep-net, 15 Oct 2025, coll. I. E. Bozdoğan (*A. biguttula* detected); Tayfursökmen (36°14′49.5″ N, 36°24′49.1″ E; 86 m a.s.l.), on *G. hirsutum*, sweep-net, 15 Oct 2025, coll. I. E. Bozdoğan (*A. biguttula* detected); Tayfursökmen (36°13′33.6″ N, 36°25′36.4″ E; 88 m a.s.l.), on *G. hirsutum*, sweep-net, 15 Oct 2025, coll. I. E. Bozdoğan (*A. biguttula* detected).

Antakya District: Arpahan (36°21′00.3″ N, 36°14′20.6″ E; 90 m a.s.l.), on *G. hirsutum*, sweep-net, 6 Oct 2025, coll. I. E. Bozdoğan (*A. biguttula* not detected).

Morphological diagnosis. Morphological terminology and diagnostic interpretation follow Xu *et al.* (2017). The examined specimens from Hatay Province (Türkiye) agree well with the morphological characteristics of *Amrasca* (*Sundapteryx*) *biguttula* (Ishida, 1913) described therein. Adult males (Fig. 2A–G) possess the typical delicate, wedge-shaped body, pale green coloration in life, and two distinct preapical black spots on the crown and forewings.

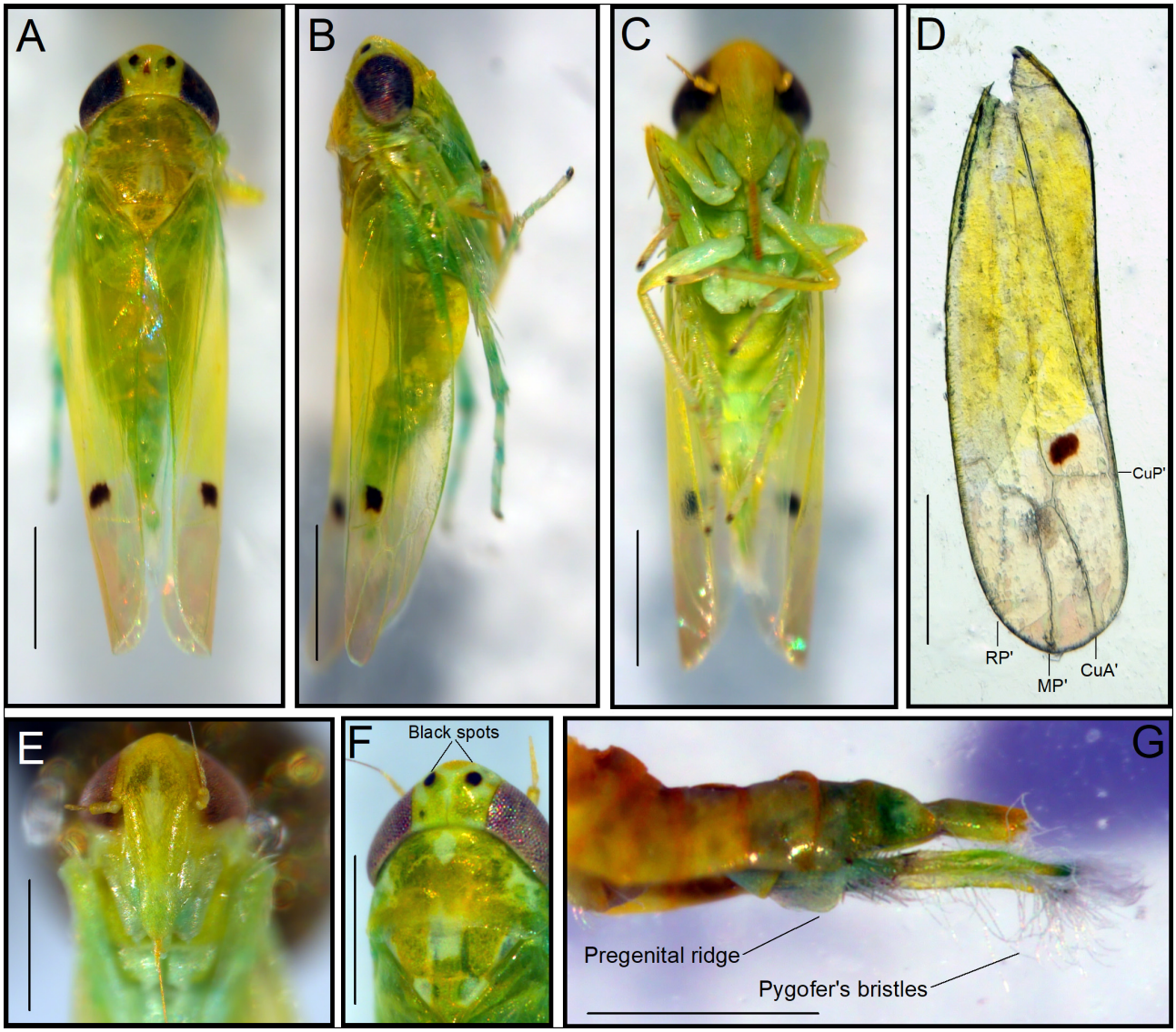

FIGURE 2. *Amrasca biguttula* (Ishida, 1913), adult male. (A) dorsal view; (B) lateral view; (C) ventral view; (D) forewing; (E) face; (F) head and thorax; (G) male abdomen, lateral view. Scale bars = 0.5 mm.

In dorsal and lateral views (Fig. 2A–C), the head is slightly wider than the pronotum, the vertex moderately produced medially, and the coronal suture short, not reaching the anterior margin. Ocelli are positioned on the anterior margin close to the compound eyes. The face (Fig. 2E) is broad with a weakly convex anteclypeus, and the pronotum is transversely striated and slightly broader than the head. The forewings (Fig. 2D) are transparent with distinct venation, showing veins MP', RP', CuA', and CuP' arising near the base of the apical cells. The subgenital plates (Fig. 2G) are triangular, narrowing distally, and bear dense rows of long setae along the lateral margins.

The male terminalia (Fig. 3A–E) exhibit the diagnostic features of *A. biguttula*: tergite VIII with an internal ridge and well-developed lateral apodemes, pygofer elongate with numerous stout setae laterally, connective Y-shaped, and aedeagus elongate, tubular, slightly curved dorsally, with a narrow preapical gonopore. The style is slender, apically curved inward. These characters correspond closely to those in the neotype description and subsequent illustrations of Xu *et al.* (2017).

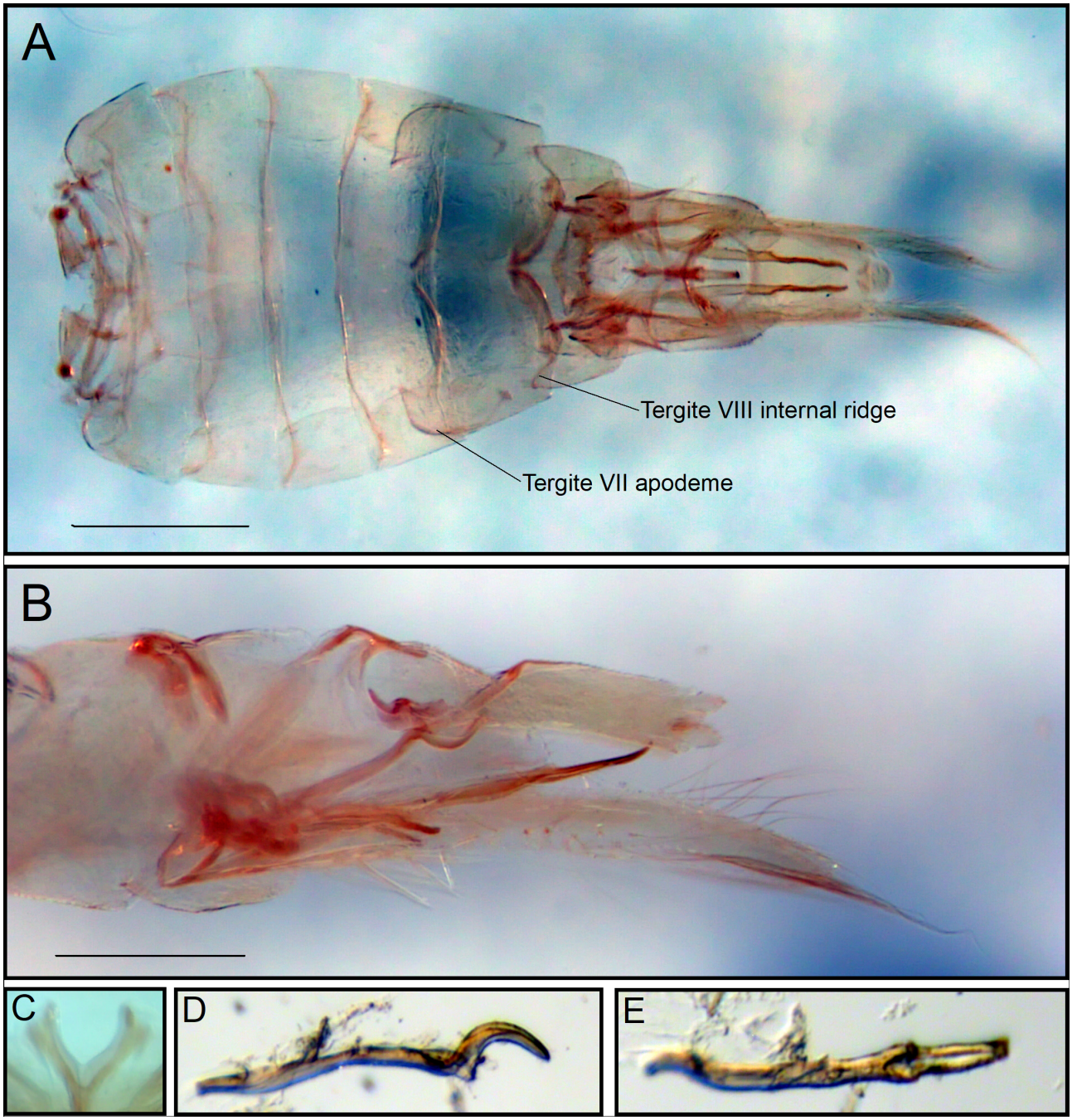

FIGURE 3. *Amrasca biguttula* (Ishida, 1913), adult male. (A) male abdomen and genitalia, ventral view; (B) abdomen and genitalia, lateral view; (C) connective; (D) aedeagus, lateral view; (E) aedeagus, ventral view. Scale bars (A, B) = 0.2 mm.

Overall, the external and internal morphological characters of the examined specimens correspond closely to the diagnostic redescription of *A. biguttula* provided by Xu *et al.* (2017), which served as the primary reference for species determination. Additional comparison with published morphological accounts from different parts of the species' native and invaded range, including Asian, African, Caribbean and American populations, shows that the observed variation in body pigmentation, forewing spot intensity and male genital structures falls within the documented intraspecific range (Sagarbarria *et al.* 2020; Cabrera-Asencio *et al.* 2023; Akonde *et al.* 2024; Esquivel *et al.* 2025; Michel & Orozco 2025). These combined assessments further substantiate the accurate identification of the Hatay specimens as *A. biguttula*.

Distribution. Japan (Okinawa), China (Anhui, Zhejiang, Hubei, Hunan, Jiangxi, Hainan, Taiwan), India, Pakistan, Bangladesh, Sri Lanka, Vietnam, Thailand, Indonesia (Java, Sumbawa), Philippines, Afghanistan, and Micronesia (Xu *et al.* 2017). Recently reported from West and Central Africa, including Côte d'Ivoire (Kouadio *et al.* 2022), Burkina Faso, Mali, Togo, and Senegal (Badiane 2023), Benin (Yarou *et al.* 2023), Niger (Akonde *et al.* 2024), and Cameroon (Jacques *et al.* 2024), where it causes damage to okra, cotton, and guinea sorrel. In the Western Hemisphere, the species was first detected in Puerto Rico (Cabrera-Asencio *et al.* 2023) and subsequently reported from Barbados (IPPC 2024), Antigua, Anguilla, St. Kitts and Nevis, and the Virgin Islands (Liburd *et al.* 2024), as well as from Martinique (Dumbardon-Martial & Pierre 2025) and multiple counties in Florida, USA (Liburd *et al.* 2024). Most recently, Michel & Orozco (2025) documented the first record of *A. biguttula* from Central America (Honduras), representing the westernmost occurrence of the species to date. Contrary to earlier assumptions, Azrag *et al.* (2025) erroneously listed the species as occurring in South America.

Remarks. The neotype of *Empoasca biguttula* was designated by Xu *et al.* (2017) to stabilize the taxonomic identity of this economically important species. It is recognized as the true "two-spotted cotton leafhopper" a major pest of *Gossypium hirsutum*, *Solanum melongena*, and other Malvaceae and Solanaceae hosts (Michel & Orozco 2025). The present study represents the first confirmed record of *Amrasca* (*Sundapteryx*) *biguttula* (Ishida, 1913) from Türkiye, extending the known western Eurasian distribution limit of the species into the eastern Mediterranean region.

Molecular identification

The partial COI sequences obtained from Hatay specimens yielded clear amplicons of approximately 658 bp. After quality trimming, one high-quality sequence was deposited in GenBank under the accession number PX453642. Identical or nearly identical COI haplotypes were obtained from all successfully sequenced specimens, and one representative sequence was deposited in GenBank (PX453642). BLAST analysis showed a 99.40–100% identity with published *Amrasca biguttula* sequences from India (e.g., PP930924, PP935748, OR004178). Alignment with reference sequences using MUSCLE revealed no insertions or deletions, and only 1–3 variable nucleotide sites were detected among all sequences, consistent with the low intraspecific COI divergence previously reported for *A. biguttula* populations (Sagar *et al.* 2014; Akmal *et al.* 2017; Sagarbarria *et al.* 2020). Phylogenetic analysis using the Neighbor-Joining method placed the Hatay isolate within a strongly supported monophyletic clade comprising *A. biguttula* sequences from India and Southeast Asia, with bootstrap values exceeding 95% (Fig. 4). The Turkish sequence clustered most closely with Indian haplotypes PP930924 and PP935748, forming a subgroup with negligible genetic distance, indicating that the Hatay population belongs to the widespread Asian lineage of the species. Sequences of related genera (*Empoasca*, *Cicadella*) and the outgroup *Diaphorina citri* (MT229895) formed distinct, well-separated clades. These molecular results fully corroborate the morphological identification and confirm that the specimens collected from cotton and eggplant fields in Hatay Province represent true *A. biguttula*, marking the first genetically verified occurrence of the species in Türkiye.

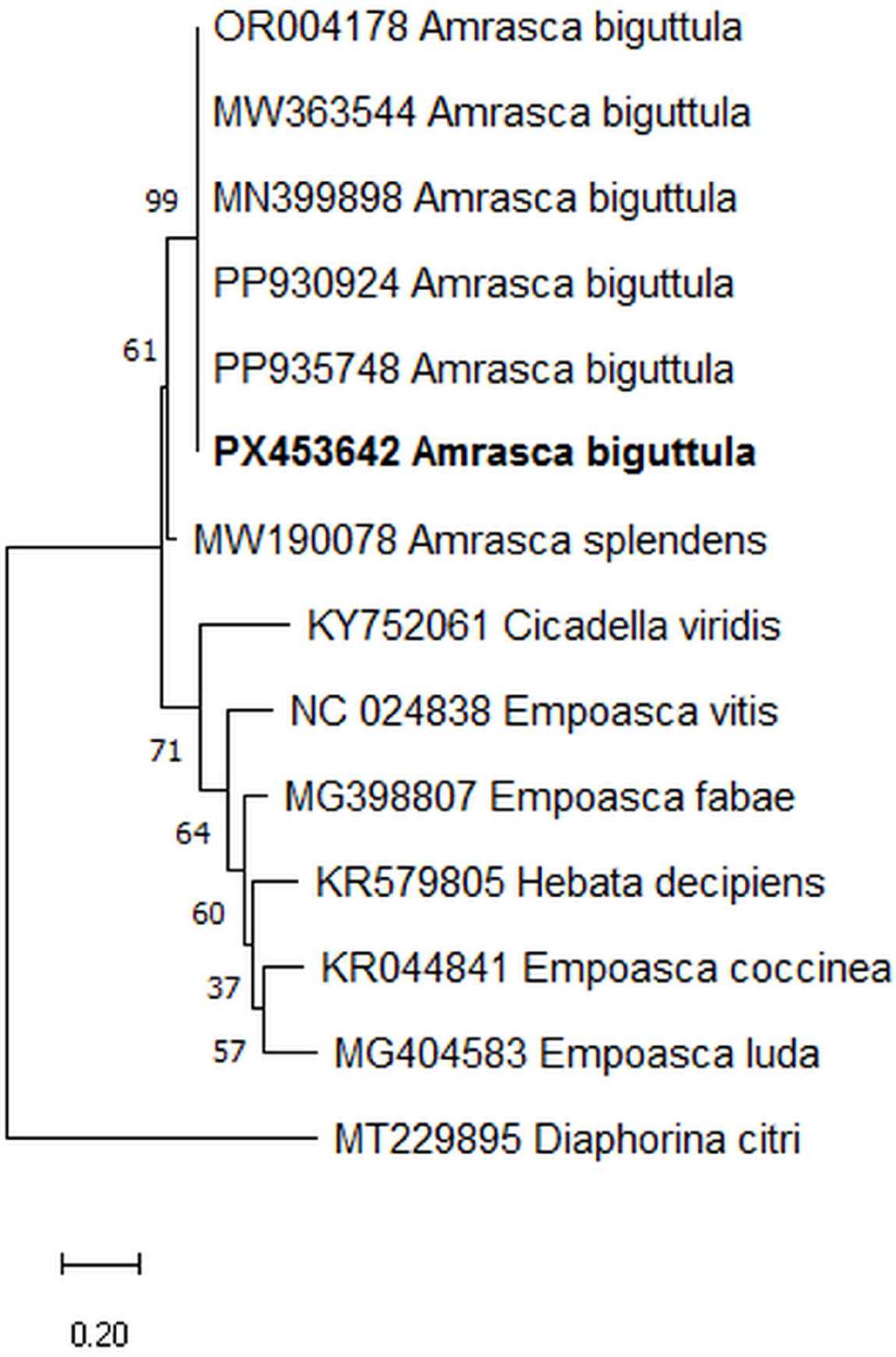

FIGURE 4. Neighbor-Joining phylogenetic tree based on partial COI sequences of *Amrasca biguttula* and related taxa. The Hatay isolate (PX453642) is highlighted in bold. Bootstrap values (1,000 replicates) are shown at nodes. The Turkish sequence clusters with Asian *A. biguttula* haplotypes, while *Diaphorina citri* serves as the outgroup.

Damage and host association

Field observations from Hatay Province showed that both adults and nymphs of *Amrasca biguttula* were actively feeding on the lower surface of cotton leaves (Fig. 5A–B). Feeding produced typical "hopperburn" symptoms (Fig. 5C), including marginal yellowing, necrosis, chlorosis, and upward curling of young leaves. These symptoms are consistent with reports from invaded cotton-producing regions across the Caribbean, Africa, and North America (Cabrera-Asencio *et al.* 2023; Jacques *et al.* 2024; Esquivel *et al.* 2025). In severely infested plants, foliage desiccation and premature leaf drop were evident, consistent with hopperburn damage caused by continuous sap extraction and toxin injection into leaf mesophyll tissues (Backus *et al.* 2005).

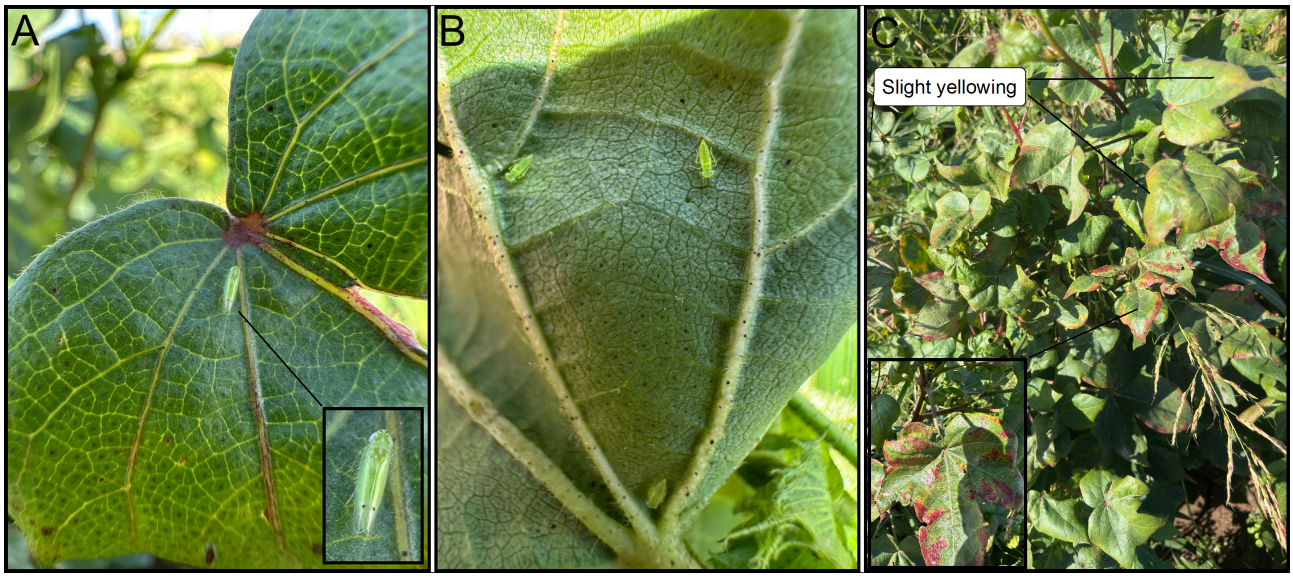

FIGURE 5. Field photographs of *Amrasca biguttula* (Ishida, 1913) in Hatay Province, Türkiye. (A) Adult feeding on a cotton leaf. (B) Nymphs on the lower surface of a cotton leaf. (C) Characteristic "hopperburn" symptoms on cotton leaves showing marginal chlorosis and necrosis.

The pest's impact on cotton production is substantial. In Asia, *A. biguttula* has been reported to reduce cotton yields by 40–60% (Azrag *et al.* 2025), while recent outbreaks in West Africa have caused 10–50% losses in affected regions (Jacques *et al.* 2024). In the U.S., Esquivel *et al.* (2025) documented rapid population build-up within four weeks of detection, with severe foliar necrosis, chlorosis, and defoliation across more than 100 counties, underscoring its high reproductive potential and capacity to reach economic injury levels swiftly. Similar yield losses have been recorded in okra and cowpea fields where the pest co-occurs (Thapa *et al.* 2019; Jaod & Nawar 2023).

Given that cotton (*Gossypium hirsutum* L.) is one of Türkiye's most valuable industrial crops, cultivated on more than 467 thousand hectares (TURKSTAT 2024) and supporting the national textile, oil and biodiesel industries, the establishment of *A. biguttula* represents a serious phytosanitary concern. The detection of both nymphs and adults on cotton and eggplant in Hatay confirms active breeding populations and local establishment. The pest is well adapted to warm-temperate climates, and the climatic conditions of eastern Mediterranean of Türkiye fall within the environmentally suitable range predicted for *A. biguttula* (Azrag *et al.* 2025). According to the climatic suitability models reported by Azrag *et al.* (2025), environmentally suitable habitats extend across the Middle East, North Africa, and southern Europe, indicating a high risk of further northward expansion. Previous studies from Hatay have already documented intensive leafhopper pressure and associated yield losses in local cotton systems (Demirel & Yıldırım 2008; Bozdoğan & Demirel 2024a,b). Continuous field monitoring, early detection programs, and region-wide biosecurity coordination are therefore essential to mitigate its potential impact on Türkiye's cotton industry and the broader Mediterranean basin.

This study provides the first verified record of *A. biguttula* from Türkiye, based on a combination of diagnostic morphology and COI sequence data. Specimens collected from cotton and eggplant fields in Hatay Province were conclusively identified and placed within the Asian lineage of the species through phylogenetic analysis. These

findings extend the known distribution of *A. biguttula* into the eastern Mediterranean and emphasize the need for continued monitoring, given the pest's expanding range and potential impact on regional cotton and vegetable production.

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