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First Record of *Melanaphis donacis* (Hemiptera: Aphididae) in Georgia[1]

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Melanaphis donacis (Passerini) (Fig. 1) can be found in Europe (France, Greece, and Maltese Archipelago), Asia (India, Iran, Iraq, and Pakistan), Africa (Morocco, South Africa, and Tunisia), North America (U.S. state of California), and South America (Chile and Argentina) (Ali et al. 2012, Adv. Biores. 3(4):66–75; Amin et al. 2019, Asian J. Agric. Biol. 7(3):381–385; Canavan et al. 2019, Bull. Entomol. Res. 109:287–299; Dudley et al. 2008, Proc. XII Intern. Symp. Biol. Contr. Weeds, CAB International, Wallingford, UK:146–152; Khaled-Gasmi et al. 2023, Ann. Appl. Biol. 182(1):101–111; Mifsud et al. 2011, Bull. Entomol. Soc. Malta 4:5–53; Mokhtari et al. 2012, Acta Entomol. Serbica 17(1/2):1–22; Ortego et al. 2004, Rev. Soc. Entomol. Arg. 63(1–2):19–30; Raychaudhuri and Banerjee 1974, Oriental Insects 8(3):365–384; Stary and Sekkat 1987, Ann. Soc. Entomol. Fr. 23(2):145–149; Tsitsipis et al. 2007, Bull. Insectology 60(1):31–38; Undurraga et al. 2020, Int. J. Agric. Nat. Resour. 47(2):117–125). This aphid was found recently on *Arundo donax* L. (giant reed) in 2023 in Mexico (Vanegas-Rico et al. 2023, Entomol. Commun. 5:ec05031). Its most common host is *Arundo donax*, but it also will colonize *Phragmites* spp. and possibly *Bambusa* spp. and *Arundinaria* spp. (Blackman and Eastop 2023, https://aphidnet.org; Raychaudhuri and Banerjee 1974; Holman 2009, Host plant catalogue of aphids: Palearctic region, Springer).

Melanaphis donacis apterous adults are pear-shaped and have a characteristic pattern of white flocculent wax that contrasts with their dark purple abdominal area (Fig. 1F) (Undurraga et al. 2020). Sexual forms have been found in southern France, but many populations worldwide are anholocyclic (Blackman and Eastop 2023; Dransfield and Brightwell 2023, https://influentialpoints.com/Gallery/Melanaphis_aphids.htm).

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Fig. 1. *Melanaphis donacis* in Tifton, GA. (A) Whole aphid, (B) anterior, (C) antennal segment III, (D) antennal segment VI, (E) posterior, (F) live aphids present on the underside of *Arundo donax* leaf, (G) leaf collar of *Arundo donax*, (H) site location of *Arundo donax* where *Melanaphis donacis* was found. Model is 5'4". Photographs A–F were of *Melanaphis donacis* collected from *Arundo donax* growing at the restaurant field in Tifton, GA. Photographs G–H are of *Arundo donax* at the same location.

Aphid damage to *Arundo donax* has been reported as minimal (Undurraga et al. 2019, Chil. J. Agric. Anim. Sci. 35(2):205–211) but virus transmission may be possible, as another *Melanaphis* species, *M. sacchari* (Zehntner), can transmit millet red leaf virus (Chan et al. 1991, Aphid-transmitted viruses and their vectors of the world, Agric. Canada Tech. Bull. 1991-3E).

Infested leaves of *A. donax* were collected on 1 December 2023 in gallon-sized Ziploc bags or microcentrifuge tubes and were transported to the USDA laboratory in Tifton GA. Aphids were removed from the leaves using a paint brush and placed in microcentrifuge tubes that contained 4 Zn-plated BBs (Daisy Outdoor Products, Rogers, AR, USA). Between samples, the paintbrush was swirled in 8.25% sodium hypochlorite and then rinsed with distilled water. The tubes were placed in the −80°C freezer until DNA extraction. To extract aphid DNA, the tubes were placed in liquid nitrogen and then ground using a Vortex Genie 2 (Daigger Scientific, Vernon Hills, IL). Samples were removed repeatedly from the liquid nitrogen for less than 10 s for grinding until a fine powder was formed. DNA was extracted using a GeneJET Plant Genomic DNA Purification kit (Thermo Fisher Scientific, Waltham,

Table 1. Collection locations of *Melanaphis donacis* in Tifton, GA.

City, State	GPS Coordinates	Description	Present	Infestation
Tifton, GA	31.451111, −83.531389	Field next to a restaurant	Alates, apterae	Heavy with multiple aphid colonies
Tifton, GA	31.490833, −83.521667	A USDA Field	4 Alates	Light and only alates were collected

MA) following the manufacturer's recommendations, except aphids were used instead of plant tissue and one elution was performed.

Using an Applied Biosystems GeneAmp PCR System 9700 thermocycler (Thermo Fisher Scientific), the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified from aphid samples obtained from *A. donax* in Tifton, GA as well as *Melanaphis sorghi* (Theobald) (Armstrong 1) and *M. sacchari* (Armstrong 48) (Harris-Shultz et al. 2022, Insects 13:416). *Melanaphis sorghi* was collected from *Sorghum bicolor* (L.) Moench grown in Matagorda Co, TX in 2013 and *M. sacchari* was collected from sugarcane (*Saccharum* sp.) in Belle Glade, FL in 2017. Armstrong 1 and 48 were obtained from Dr. Scott Armstrong, USDA-ARS. The primers, LCO1490 and HC02198, used had been described previously (Folmer et al. 1994, Mol. Mar. Biol. Biotechnol. 3(5):294–299). The 20 μL PCR reaction consisted of 4 μL of 5x Phusion HF buffer (Thermo Fisher), 1.6 μL of 2.5 mM of dNTP mix, 1 μL of 5 μM LCO1490 primer, 1 μL of 5 μM of HC02198, 0.2 μL of Phusion DNA polymerase, 11.2 μL of water, and 1 μL of stock DNA (9.8–140 ng/μL). Two controls were also amplified where the stock DNA was replaced in the reaction with water. The thermocycler conditions consisted of an initial denaturation of 98°C for 30 s, followed by 34 cycles of 98°C 7s, 55°C for 20 s, and 72°C for 30s. A final elongation was performed at 72°C for 7 min. Six μL of PCR product and 2 μL of 5x Green GoTaq Flexi buffer was loaded onto a 1% agarose gel to ensure a ~700 bp fragment was amplified in the sample wells. To remove primers and nucleotides, the 14 μL of remaining PCR product was treated with 5.6 μL of ExoSAP-IT (Thermo Fisher Scientific) and placed in a thermocycler at 37°C for 4 min and 80°C for 1 min. For sequencing, 5 μL of each ExoSAP-IT treated PCR product was added to 5 μL of 5 μM of LCO1490 or 5 μL of 5 μM of HC02198 and samples were shipped out for sequencing (Eurofins Genomics, Louisville, KY). Sequences were trimmed and cleaned using Sequencher 4.10.1.

Melanaphis donacis was found at two locations in Tifton, GA (Table 1). One was behind the parking lot of a local restaurant [31.451111, −83.531389] (restaurant field), and the other was in an experimental USDA field [31.490833, −83.521667] (USDA field). Aphids were collected on *A. donax* on the leaf underside, and alatae, and apterae were visible at the restaurant field, whereas only 4 alatae were observed at the USDA field (Table 1). No ants were seen attending aphids at the time of collection, but lacewing eggs (genus, species unknown) were present next to a colony at the restaurant location. The identity of these aphids was determined using morphological and molecular methods.

Some of the aphids that were collected from the restaurant field were placed in a microcentrifuge tube filled with 70% ethanol for morphological identification. These specimens were mailed to Dr. Susan Halbert, Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) in Gainesville, FL for identification. Six adults were cleared and mounted in Canada balsam. Specimens are deposited in the Florida State Collection of Arthropods (FSCA). Morphological identification was completed only on aphids at the restaurant field because large numbers of aphids were present at that location. Morphological identification determined the aphids to be *Melanaphis donacis* (Fig. 1).

Sequencing of the COI from both locations revealed that both sequences were identical and were most similar to the COI from *Melanaphis donacis* from Pakistan (Accession MN319722) and Europe (Accession KF639522-country not given) with a 96% coverage, E-value=0, and an identity of 99.85%. A single SNP at 434 bp on the *M. donacis* Tifton, GA sequence was seen between the aphids from Tifton, GA and the sequences from Pakistan and Europe. The sequence obtained from *M. donacis* from Tifton, GA was deposited in GenBank (OR944927). Additionally, the Florida Department of Agriculture and Consumer Services, Division of Plant Industry Molecular Diagnostic Laboratory's sequencing of the COI generated identical results (PP091623).

There are 6 species of *Melanaphis* known now from the southeastern USA. All are adventive from the Old World except *Melanaphis arundinariae* (Tissot), which is known only from collections in the early 1930s in Florida. Other *Melanaphis* species in the southeastern states include *Melanaphis bambusae* (Fullaway), *Melanaphis sacchari*, *Melanaphis sorghi*, and *Melanaphis sorini* Halbert & Remaudière. *Melanaphis donacis* can be distinguished from all these other *Melanaphis* by the short processus terminalis of antennal segment VI (Fig. 1D). Prior to the appearance of *M. donacis*, only *Hyalopterus pruni* (Geoffroy) was found commonly on *A. donax*. *Hyalopterus pruni* colonies consist of light green or pink elongate aphids and thus can be distinguished easily from *M. donacis*.

Melanaphis donacis is probably native to the Middle East and Central Asia. Data from morphological and DNA analysis show that *Melanaphis donacis* is present on *A. donax* in Tifton, GA. Previously, this species only had been reported to be in California in the USA. We suspect that further examination of *A. donax* throughout the southeastern US may identify additional aphid colonies.

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