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THE NEOTYPE OF THE COTTON APHID (HEMIPTERA: APHIDIDAE: *APHIS GOSSYPII* GLOVER 1877)

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Abstract.—We fix and describe the **neotype** of the cotton aphid, *Aphis gossypii* Glover, and publish its DNA barcode.

Key Words: DNA barcode, type specimen, *Aphis circezandis*, *A. frangulae*, *A. oestlundi*

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Aphis gossypii Glover is one of the most biologically diverse and economically important aphid species (Blackman and Eastop 2007; Deguine et al. 2007). Ebert and Cartwright (1997) list 90 plant families that have been recorded as hosts of *A. gossypii* and 66 host plant species where aphids have reached numbers "requiring human intervention." *Aphis gossypii* has been recorded as transmitting over 75 plant viruses (Chan et al. 1991). Due to this aphid's economic importance and ubiquity, *A. gossypii* has been described and redescribed many times (Favret 2010, Remaudière and Remaudière 1997). A thorough review of its biology is presented by Ebert and Cartwright (1997). Due to the complex taxonomic status of the species (and/or species complex), we propose a fixation of a primary type specimen. Furthermore, having acquired fresh type material, we take the opportunity to sequence the majority of the mitochondrial cytochrome oxidase C subunit 1 gene. We here publish this sequence, the DNA barcode, and compare it to other published barcodes of *A. gossypii*.

We refer to Article 75.3 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999) in presenting a case for the fixing of a neotype for *Aphis gossypii* Glover.

75.3.1. a statement that it is designated with the express purpose of clarifying the taxonomic status or the type locality of a nominal taxon.

Because it is an important agricultural pest with a cosmopolitan distribution, the species has acquired 42 available synonyms (Favret 2010, Remaudière and Remaudière 1997). In the second half of the 20th Century, an infrequently used name, *A. circezandis* Fitch 1870, was found to have synonymic priority over *A. gossypii*. An appeal was made to the International Commission for Zoological Nomenclature (ICZN) for suppression of *A. circezandis* in favor of *A. gossypii* (Russell 1968). The suppression was approved, but for the sake

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of nomenclatural clarity, Russell was asked to designate a neotype for *A. gossypii* (Melville 1979; correspondence letters in file at the Systematic Entomology Laboratory, Beltsville, MD). A neotype has not been designated until now.

Aphis gossypii is considered one of the most complex aphid taxonomic entities. Some populations "regarded as *Aphis gossypii* may be functioning as distinct species" (Blackman and Eastop 2006) and "it may be necessary to consider separate populations of *A. gossypii* as distinct taxonomic entities" (Blackman and Eastop 2000). There are several aphid species that are very similar morphologically (e.g., Voegtlin et al. 2004) and *A. gossypii* is sometimes treated as a subspecies of *A. frangulae* Kaltenbach 1845, especially in Europe (e.g., Heie 1986).

Aphis capsellae Kaltenbach 1843 is often considered a subspecies of *A. gossypii* (Remaudière and Remaudière 1997, Favret 2010) but has priority over it. The nominotypical subspecies should thus be *A. capsellae* ssp. *capsellae* and *A. gossypii* should properly be known as *A. capsellae* ssp. *gossypii*. Furthermore, *A. capsellae* and *A. sedi* Kaltenbach 1843 are both likely synonyms with priority over *A. gossypii* (Cocuzza et al. 2008), and *A. solanina* Passerini 1863 and *A. convolvulicola* Ferrari 1872 are themselves already listed as synonyms of, but with priority over, *A. gossypii* (Favret 2010, Remaudière and Remaudière 1997). Aphid nomenclature regarding these names should be brought into compliance with the International Code of Zoological Nomenclature (ICZN 1999) either 1) by using the oldest name for the nominal species, i.e., *A. capsellae*, or 2) by petitioning the ICZN for suppression of the older names in favor of *A. gossypii*.

Finally, on top of the already extensive literature on the species is a growing body of molecular analyses and diagnostics, especially with regard to distinguishing cryptic species (e.g., Foottit et al. 2008; Cocuzza et al. 2008, 2009; Carletto et al. 2009). With so much taxonomic and nomenclatural attention being paid to this one, or complex of several, problematic species, and the certainty of important future developments, we think it expedient to fix a primary type.

75.3.2. a statement of the characters that the author regards as differentiating from other taxa the nominal species-group taxon for which the neotype is designated, or a bibliographic reference to such a statement. There is no shortage of literature dealing with the diagnosis of *Aphis gossypii* (Blackman and Eastop 2000, 2006; Heie 1986 [as *A. frangulae gossypii*]; Kono and Papp 1977; Lampel and Meier 2007; Nieto Nafría et al. 2005; Stoetzel et al. 1996; Voegtlin et al. 2003, 2004).

75.3.3. data and description sufficient to ensure recognition of the specimen designated. See the full text description and images below.

75.3.4. the author's reasons for believing the name-bearing type specimen(s) (i.e., holotype, or lectotype, or all syntypes, or prior neotype) to be lost or destroyed, and the steps that had been taken to trace it or them. Townend Glover, valuing drawings above actual specimens (Russell 1968), did not designate types for the cotton aphid. In fact, he did not even give his species a name during his first several descriptions (Glover 1855, 1856; Glover in Newton 1866); it was not until 1877 that we date the name *Aphis gossypii*. No types have ever been fixed for the species.

75.3.5. evidence that the neotype is consistent with what is known of the former name-bearing type from the original description and from other sources; however, a neotype may be based on a different sex or life stage, if necessary or desirable to secure stability of nomenclature.

Although there never was an original name-bearing type, the proposed neotype fits well within the morphological and biological range of the original descriptions (Glover 1855, 1856, 1877, Newton 1866).

75.3.6. evidence that the neotype came as nearly as practicable from the original type locality [Art. 76.1] and, where relevant, from the same geological horizon or host species as the original name-bearing type (see also Article 76.3 and Recommendation 76A.1). The original literature (Glover 1855, 1856, 1877, Newton 1866) does not mention a collection locality from which Glover based his descriptions, but the plant host was cotton, *Gossypium hirsutum* L., and the original material came from an American state of the Deep South (Alabama, Georgia, Mississippi, or South Carolina; Russell 1968). We acquired fresh material of *A. gossypii* on cotton from South Carolina.

75.3.7. a statement that the neotype is, or immediately upon publication has become, the property of a recognized scientific or educational institution, cited by name, that maintains a research collection, with proper facilities for preserving name-bearing types, and that makes them accessible for study. The neotype is deposited at the United States Museum of Natural History, in the National Aphid Collection, Beltsville, MD. It has been databased as specimen "USNM-ent 396854." The lectotype of *A. circezandis* is also located in the National Aphid Collection, specimen "USNM-ent 399255."

Materials and Methods

Aphids were collected by hand on the underside of leaves of cultivated cotton in South Carolina. DNA was extracted non-destructively using Qiagen extraction kits (Favret 2005) and the resultant cleared specimens mounted to microscope slides in Canada balsam. Species identity was confirmed morphologically using a published key (Blackman and Eastop 2000) and comparison with specimens in the United States National Museum of Natural History Aphid Collection (Beltsville, MD).

Historically, insect molecular taxonomy has used the 3′ end of the CO1 gene, whereas DNA barcoding uses the 5′ end. Given the importance of the type's barcode, and given the availability of universal CO1 primers and ease of sequencing, we sequenced the majority of the gene. We used universal primers C1-J-1718 and TL2-N-3014 (Simon et al. 1994), and LepF and LepR primers (Foottit et al. 2008) for both PCR amplification and sequencing using standard protocols (Favret and Voegtlin 2004, Favret 2005, Favret 2009) and an annealing temperature of 55 °C.

Eleven specimens were barcoded and a single apterous female vivipara specimen was selected as neotype. All specimens are deposited in the Aphid Collection of the U.S. National Museum of Natural History, Beltsville, MD.

To compare the neotype's barcode with currently available *A. gossypii* barcodes, we obtained all 5′ CO1 sequences (i.e., barcodes) classified as *A. gossypii* from GenBank (see accession numbers in Figure 1), as well as barcodes of two closely-related species, *Aphis frangulae* Kaltenbach (Carletto et al. 2009) and *Aphis oestlundi* Gillette (Foottit et al. 2008). We aligned all 98 sequences with ClustalX (Larkin et al. 2007) (no indels were needed, although a likely human transcription error at the far 5′ end of DQ499026 was removed) and compared the pairwise level of sequence divergence. To place the neotype diagrammatically within the genetic variation of the species, we performed nested clade analysis (Templeton et al. 1995, Templeton 1998) on the aligned CO1 sequences using TCS (Clement et al. 2000).

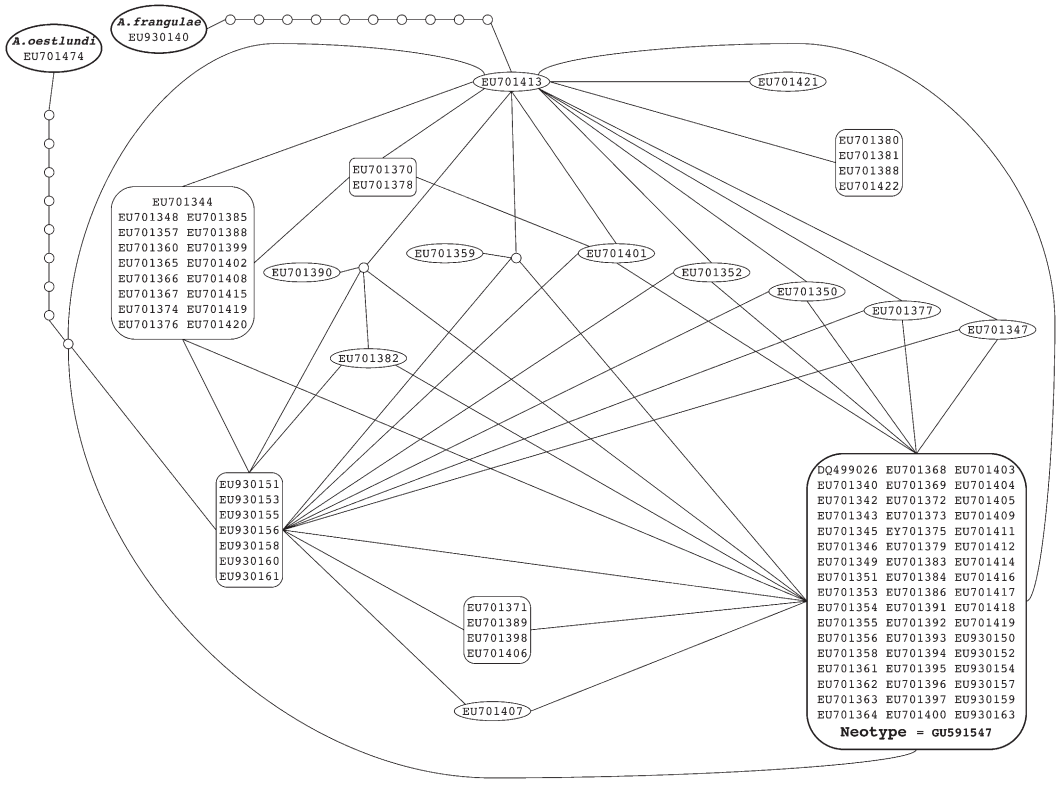

Fig. 1. Haplotype network of all available DNA barcodes of *Aphis gossypii* and a single barcode of each of two closely related species, *A. frangulae* and *A. oestlundi*. The distance between each node represents a single base difference. Ovals contain the respective GenBank accession numbers.

The neotype examination and measuring were made using AxioVision 4.6 imaging and measuring software (Zeiss, Göttingen, Germany) and a Zeiss Axio Imager M1 microscope, lengths were recorded in micrometers (μm), and important morphological characteristics drawn by hand with reference to digital images and the actual specimen.

Results

All eleven specimens provided identical barcodes. The CO1 sequence of the neotype, including the 5′ barcoding region, 1483 nucleotides in length, was submitted to GenBank with accession number GU591547. Nested clade analysis of all 98 sequences indicated that no two *A. gossypii* barcodes were distinct by more than three nucleotide differences, with a total of 17 different haplotypes (Fig. 1). The neotype's barcode was one of 52 sequences of the same haplotype. The two closely related species, *A. frangulae* and *A. oestlundi*, were 11 and 10 base substitutions away, respectively, from the nearest *A. gossypii* barcode, and 12 and 10 substitions from the neotype's barcode.

Having confirmed the identity of our specimens with both morphological and molecular means, we here fix and describe specimen 396854 of the Entomology Department of the United States National Museum of Natural History as the *A. gossypii* neotype. Most morphological terms, structures, and measurement parameters (in μm) are adapted from Blackman

and Eastop (2006). Some measurements are recorded for both the left (ls) and right sides (rs) of the slide-mounted specimen.

Aphis gossypii Glover neotype

Apterous viviparous ♀ (Fig. 2), USA: South Carolina, Calhoun County, 33.721° latitude, -80.654° longitude, on *Gossypium hirsutum* L., 15.viii.2009, C. Favret (US NMNH Entomology specimen 396854).

Description.—Body length 1506 (Fig. 2A). *Head* (Fig. 2B): width through eyes at triommatidia 354; darker than rest of body; pigmentation and faint dorsal reticulation; antennal tubercles undeveloped; tips of dorsal head setae blunt; antenna (Fig. 2C) shorter than body, without secondary sensoria; longest seta on antennal segment (a.s.) III less than half the width at its widest point; a.s. I, II, distal V, base of VI, and distal terminal process with darker pigmentation; a.s. I 50(rs), 56(ls); a.s. II 41(ls), 44(rs); a.s. III 162 (ls), 170(rs); a.s. IV 126(ls), 132(rs); a.s. V 117(ls), 119(rs); base of a.s. VI 82(rs), 88(ls); terminal process of a.s. VI 228 (ls), 233(rs); rostrum extending to mesocoxae; rostral segments IV+V 106 with 2 accessory setae (Fig. 2E). *Thorax:* dorsolaterally with darkened reticulations (Fig. 2D); profemur 226(ls), 241(rs); protibia 425(rs), 440(ls); protarsus II 68 (rs), 74(ls); mesofemur 253(ls), 255(rs); mesotibia 470(ls), 771(rs); mesotarsus II 78(ls), 80(rs); metafemur 326(rs), 331 (ls); metatibia 617(rs), 623(ls); metatarsus II 86(ls), 92(rs); dorsal mid-tibial setae slightly longer than half the width of the tibia; tarsus II setal formula 2-2-2. *Abdomen:* with faint dorsal reticulations; with ventral lines of small spicules; dorsal setae stout with blunt tips; dorsum of segment VIII with 4 setae; ventral setae long and acuminate, nearly 3.5 times longer than dorsal setae; genital plate with 3 anteriomarginal and 7 posteriomarginal setae (Fig. 2H); anal plate with 12 setae; siphunculus (Fig. 2F) 245(rs), 249(ls), scabrous, dark, tapering gradually from base to apex; cauda (Fig. 2G) 154 long, 80 wide at base, dusky, tongue-shaped, with 2 pairs of curved lateral setae.

Discussion

Aphis gossypii was described from cotton grown in the southeastern United States but its origin may be eastern Asia (Blackman and Eastop 2006). Taxonomic and nomenclatural norms suggest that a neotype be described from the type locality, but it is noteworthy that the 3′ half of the neotype's barcode is identical to a sequence from Nanjing Province, China (GenBank Accession EF640165). This latter sequence does not include the 5′ barcoding region so was not part of the larger molecular analysis.

Foottit et al. (2008) obtained a global and extensive set of barcodes for the cotton aphid and found 0.62% or less sequence divergence within the dataset. This figure does not change with the addition of the neotype's barcode which is suitably placed within the range of genetic variation of *A. gossypii*.

With a known prevalence of misidentifications (Vilgalys 2003) and lack of voucher specimens, it is unfortunate that one cannot confirm the true identity of the vast majority of deposited DNA sequences. We would therefore consider suspect the identity of any barcodes that do not fall within the 0.62% sequence divergence published by Foottit et al. (2008), where vouchers (albeit not actual barcoded specimens) are available. In the current case, however, there are no barcodes identified as belonging to *A. gossypii* in GenBank outside the 0.62% range. This low level of genetic divergence supports the hypothesis that our current concept of *A. gossypii* makes for the single most diverse aphid species "in

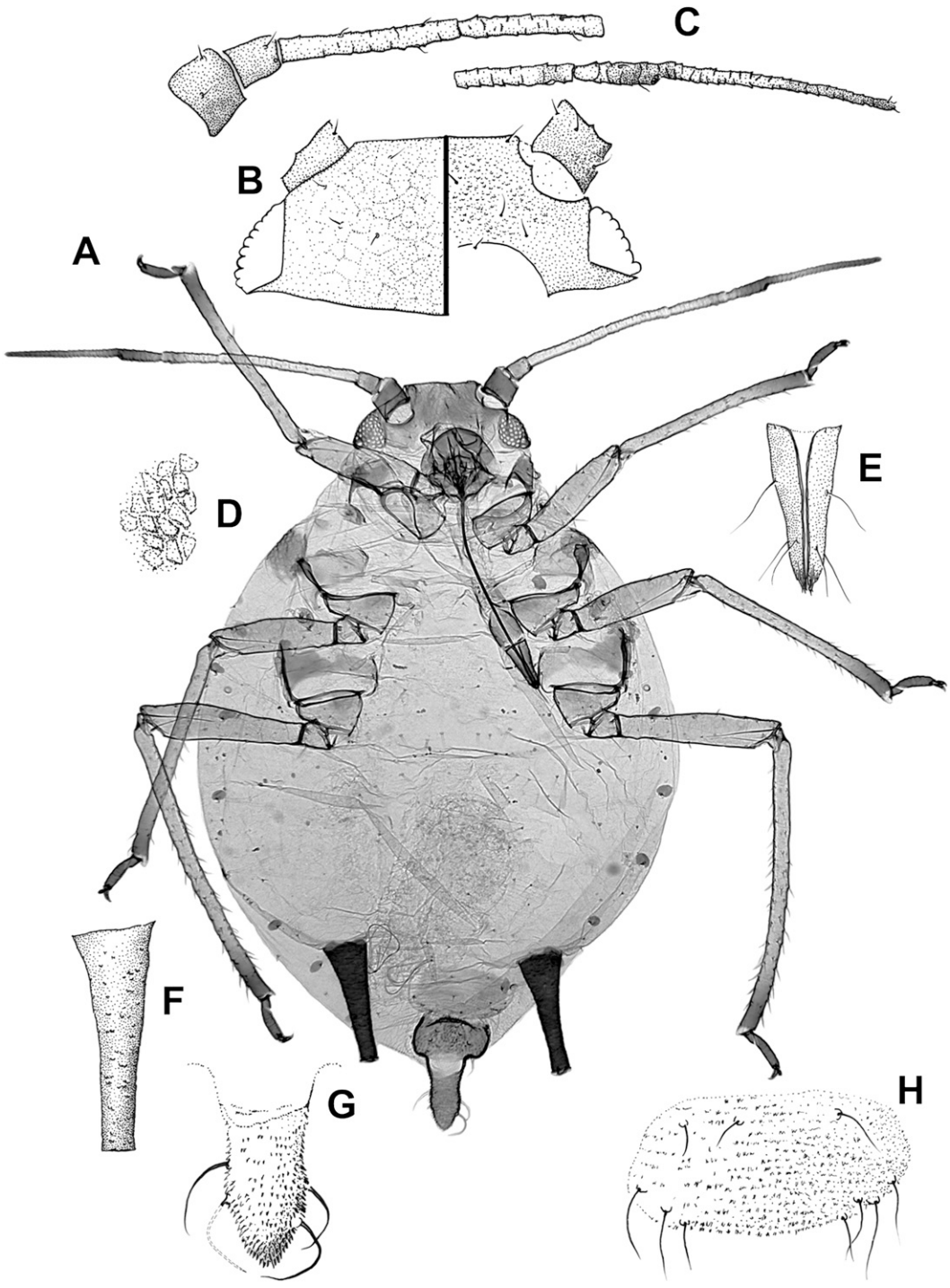

Fig. 2. Neotype photomicrograph and line drawings of *Aphis gossypii*. A, Entire specimen. B, Head (left half dorsal, right half ventral). C, Antenna. D, Thoracic reticulation. E, Ultimate rostral segments. F, Siphunculus. G, Cauda, (dashes represent likely position of broken seta). H, Genital plate.

terms of host relationships, life cycle, and geographic range" (Blackman and Eastop 2007).

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