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Nephila clavata L. Koch, the Joro Spider of East Asia, newly recorded from North America (Araneae: Nephilidae)

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ABSTRACT

Nephila clavata L. Koch, known as the Joro spider and native to East Asia (Japan, China, Korea, and Taiwan), is newly reported from North America. Specimens from several locations in northeast Georgia were collected from around residential properties in Barrow, Jackson, and Madison counties in late October and early November 2014. These are the first confirmed records of the species in the New World. Our collections, along with confirmed images provided by private citizens, suggest the Joro spider is established in northeast Georgia. Genomic sequence data for the COI gene obtained from two specimens conforms to published sequences for *N. clavata*, providing additional confirmation of species identity. Known collection records are listed and mapped using geocoding. Our observations are summarized along with published background information on biology in Asia and we hypothesize on the invasion history and mode of introduction into North America. Recognition features are given and photographic images of the male and female are provided to aid in their differentiation from the one native species of the genus (*Nephila clavipes*) in North America.

Keywords: Araneae, Nephilidae, non-native, Georgia, description, diagnosis, distribution, new record, citizen science, COI

Introduction

Golden orb-web spiders of the genus *Nephila* are pantropical in distribution, are reported to exhibit pronounced sexual dimorphism with the females being conspicuously colorful and considerably larger than the males (Su et al. 2011), and are noted for their exceptionally large, impressive webs. Up until recently, the genus *Nephila* has been variously positioned by authors in the families Araneidae and Tetragnathidae. Recent morphological, behavorial, and molecular evidence support the elevation of the subfamily Nephilinae to family rank (Harvey et al. 2007; Kuntner, 2006; Kuntner et al. 2013; Su et al., 2011).

A single species of the genus, *N. clavipes* (L.), occurs in the United States. In this paper, we provide the first collection records and photographic evidence confirming the occurrence of a second species of the genus - the Joro spider, *N. clavata* - in North America. We also give a diagnosis and description to facilitate its recognition in the Nearctic fauna, and review key literature on biology and habits in its native range.

MATERIALS AND METHODS

In late September 2014, one of us (WH) made the discovery of a large, extremely colorful, but unknown female spider in an expansive web spun between upper tree branches about 2.5 meters above-ground and approximately 20-25 meters from the back door of the home where WH lives in Madison Co. (Colbert), GA. He took the spider down from the web, photographed it using a cell phone camera, and sent the images to the Department of Entomology at the University of Georgia. Using an Internet search in his attempt to identify the spider, he concluded that this might be the Joro spider of East Asia. The spider's

identity was confirmed by ERH using taxonomic references on Asian spiders (cited herein), examining and comparing the male genital structures (palpal organ) with that illustrated in the primary literature, and by comparing specimens in hand with images posted on the Internet for this eastern Palearctic species.

This remarkable discovery prompted us to find a way to determine if other people in northeast Georgia had seen or encountered this attractive orb-weaving spider. Almost as soon as a press release appeared in the local newspaper in late October (Available at http://onlineathens.com/uga/2014-10-30/madison-county-man-captures-spider-never-seen-north-america), highlighting this first-time detection in North America with a photographic image of the spider, we heard almost immediately from several concerned citizens throughout a 3-county area of northeast Georgia. Responding E-mails included attached images of the spiders they sighted around their primary residence and property.

Over the course of the next week to 10-days, we had confirmed sightings from at least 9 locations (see locality data under North American records). With the exception of only a few sightings, we were able to collect specimens (females) from all locations. Knowing that spiders would soon disappear because of the approaching cold weather, we collected as many specimens as possible for preservation and for further molecular studies. On one occasion at a small community park (Braselton Park) in Braselton, GA (November 4), we (ERH and BJF) collected specimens (2 females, 2 males) in webs around the perimeter of a tennis court and along the wooded edge of the park. These are the only 2 males we have found to date.

A genetic analysis involved the sequencing of specimens (n=2 females) from Jackson (GMNHTC 12242) and Madison (GMNHTC 12241) counties. Genomic DNA was extracted from single spider legs previously preserved in 95 percent ethanol, using a Chelex^{TM}100 Resin and proteinase-k solution, incubated overnite at 55°C(Casquet et al. 2012). The cytochrome C oxidase subunit I (COI) mitochondrial gene was amplified by polymerase chain reaction (PCR) using the primer combinations of LCO1490: 5'-GGTCAACAAATCATAAAGATATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAA ATCA-3' (Folmer et al. 1994, Su et al. 2011), and recovered a 639 base pair fragment after sequencing. PCR reactions followed conditions reported in Wares et al. (2001). PCR products were assayed by electrophoresis using a 1.0 percent Hagarose gel and sequencing was performed by Macrogen (Macrogen, USA; available at https://www.macrogenusa.com/). Sequence files were aligned using CodonCode Aligner (available from http://www.codoncode.com/aligner), and Geneious version 8.03 (created by Biomatters, available at http://www.geneious.com/) was used to visualize sequence data and compared with published sequences for Nephilidae.

Voucher specimens of *N. clavata* are deposited in the Collection of Arthropods, Georgia Museum of Natural History (UGCA), and specimens sequenced are also catalogued in the Georgia Museum of Natural History Genomic Collection (GMNHTC) University of Georgia, Athens, GA, and an ArcGIS map of collection locations of *N. clavata* in northeast Georgia is provided.

RESULTS

Nephila clavata L. Koch
Figs. 1A-D, 2

Annotated synonymy:
Nephila clavata L. Koch 1878: 741.
Nephila limbata Thorell 1898: 335.
Nephila obnubila Simon 1906: 308.
Nephila clavatoides Schenkel 1953: 27.
Nephila clavata cavaleriei Schenkel 1963: 134.

Systematics. Golden orb-web spiders of the genus *Nephila* are found throughout tropical and subtropical regions of the world (Kuntner et al., 2013). The genus is comprised of 38 species and subspecies (World Spider Catalog, 2014). *Nephila clavipes* (L.) is the only species of the genus known to occur in the Western Hemisphere. It is found throughout Florida, the West Indies, as far north as North Carolina, across the Gulf States, through Central America, and into South America as far south as Argentina (Weems and Edwards, 2004). In the United States, it is often referred to as the "banana spider" or the "golden silk spider."

Nephila clavata is called the Joro spider in Japan (Japanese name: jorō-gumo). "Jorō-gumo" is a legendary creature in Japanese folklore that can change its appearance into that of a beautiful, seductive

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woman (brief discussion in Wikipedia posted at http://en.wikipedia.org/wiki/Jor%C5%8Dgumo. In Korea, it is referred to as the "Mudang" spider (Korean name: mudang gumi) which translates into "shamen" or "fortune teller" spider (brief discussion in "Whatsthatbug.com" posted at http://www.whatsthatbug.com/2005/12/22/ mudang-spider-of-korea/).

Species diagnosis. *Nephila clavata* females can be easily distinguished from those of *N. clavipes* are their unique coloration (opisthosoma bright yellow, when alive, with broad, horizontal bluish-green bands on the dorsum, a large red marking on the venter, and long, black legs with yellow-orange bands). In contrast, the female of *Nephila clavipes* can be readily recognized by the color pattern alone - the silvery carapace, yellow spots on a dull orange to tan cylindrical opisthosoma, brown and orange-banded legs, plus the exaggerated hair brushes on the tibial segment of legs I, II, and IV (Weems and Edwards, 2004). Among other large orb-weaving spiders rivaling *N. clavata* in size, only the yellow and black Argiope or yellow garden spider, *Argiope aurantia* (F.), could be confused. However, it differs in body coloration (opisthosoma with striking yellow or orange markings on a black background) and in web architecture (circular web with a dense zigzag of silk, known as a stabilimentum, in the center).

Species description. [adapted from Zhu and Zhang (2011) and Tanikawa (2007)].
Female (Figs. 1A-C).
Length: 17-30 mm.
Prostoma: Carapace dark brown with dense, golden to silvery-white vestiture. Sternum: Dark brown with a trapezoidal yellow spot in the median area of the anterior half.
Legs: Black except for yellow-orange annulations at middle of tibia and at apical two-thirds of femur. Base of metatarsus black in some specimens while yellowish at extreme base in others; tarsus varies from black to slightly orangish.
Palpi: Yellow-orange, with terminal segment black apically.
Opisthosoma: Ovoid-cylindrical. Dorsum (Fig. 1A) bright yellow (when alive) with five narrow to broad bluish-green horizontal bands. Venter (Fig. 1C) with anterior half marked with irregular black-brown oblique stripes; posterior half with two broad, oblique, red markings anterior to the spinnerets.
Male (Fig. 1D).
Length: 4-8 mm.
Prostoma: Light brown with two dark brown longitudinal bands on both sides.
Opisthosoma: Elongate-oval. Dorsum anteriorly greenish-brown with two yellow longitudinal stripes on both sides of dark brown median line.
Palpal organ: Described and illustrated by Feng (1990: 96, Figs. 2-3); Zhu, Song, and Zhang (2003: 76, Fig. 32, I, J); Zhu and Zhang (2011: 194, Fig. 136, I, J); and Tanikawa (2007: 95, Figs. 764, 766).
Color images of the female, dorsal and lateral aspects, and the male are provided in Yaginuma (1960: 67, Plate 29, Fig. 163).

Distribution. *Nephila clavata* is found throughout Japan (except the island of Hokkaido), Korea, China, and Taiwan (Kim, Kim, and Lee, 1999). The World Spider Catalog (2014) lists the species as occurring from "India to Japan."

Biology. The following account of the biology and seasonal history is summarized from published Asian literature and Internet sites. *Nephila clavata* is univoltine in Japan. Eggs overwinter and spiderlings emerge from the egg cocoons in early June in central Japan (Miyashita, 1986, 1990; Miyashita and Hayashi, 1996). Males reach maturity by late August and females become sexually mature in September and early October. After mating, oviposition occurs from mid October to November resulting in the production of only a single egg sac (Miyashita and Hayashi, 1996). Adult males abandon webs after maturity and cohabit with females on their webs (Miyashita, 1994). Fecundity varies considerably among individual females, with a strong correlation between female body size and fecundity (Miyashita, 1986). Females achieve their greatest body weight by late October (Miyashita, 1990).

According to Kim, Kim, and Lee (1999), this species inhabits mountainsides, fields, or is found in urban and non-urban woodland sites in its native habitat. In Japan, spiders are commonly found throughout lowland forests and can also inhabit building spaces in the vicinity of small urban woods (Miyashita, 1990) where there is a high degree of human disturbance.

Females produce large, golden-yellow, basket-shaped webs between tree branches. Females oviposit between 400-1500 eggs in a single egg sac (posted on Wikipedia at http://en.wikipedia.org/wiki/Nephila_clavata; accessed 19 November 2014), inside a densely woven silk cocoon that is attached to the bark of trees, on leaves, or upon other human-made structures. Harvey, Austin, and Adams (2007) describe

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the production of a non-viscid, dense, silk platform upon which an egg sac is deposited, followed by the placement of a thick layer of bright yellow, loose, flocculent or looped silk over the egg mass. Immature spiders spin their own webs. In Korea, spiders are still active until late November. All adults die off with the onset of winter.

In northeast Georgia, our observations agree with that previously described in the Asian literature. Large, mature females were first observed beginning in late September and persisted until mid November when temperatures began to cool significantly. Most spiders were found in large webs attached to the exterior of homes near porch lights, on wooden decks, or among shrubs and flowering bushes near to homes. At one location in early November, spiders were found in webs attached to small trees and high weeds along a wooded area of a small city park (Braselton Park, Braselton). Here we also found two males in a web with a single female. At one Braselton home site, we found an egg mass, covered by a dense cocoon of silk and guarded by a female, attached to vinyl siding of a home.

North American records (Fig. 2)

Specimens, and/or images, examined.

UNITED STATES: GEORGIA: *Barrow Co*., Hoschton (1033 City Market Street), 31-X- 2014, C. Robbins (specimens, images) [34.089846, -83.8266]. *Jackson Co*., Braselton (48 Ridge Way), 31-X-2014, R. Barbani (specimens, images) [34.138962, -83.798877]; Braselton, Braselton Park (Harrison Street), 4-XI-2014, GMNHTC 12242, E. R. Hoebeke and B. J. Freeman (specimens) [34.1072909, -83.7638141]; Braselton (120 Ashwood Lane), 9-XI-2014, K. Youngblood (image only) [34.142209, -83.7564699]; Hoschton (14 Jacob Drive), 4-XI-2014, C. Glick (image only) [34.064256, -83.693733]; Hoschton (31 Otter Lane), 4-XI-2014, K. and J. Howell (specimen, images) [34.093613, -83.747015]; Jefferson (580 Colonial Court), 4-XI-2014, C. Hamilton (specimen, images) [34.097902, -83.6953489]. *Madison Co*., Colbert (124 Circle D Drive), 4-X-2014,GMNHTC 12241, W. Huffmaster (specimen, images) [34.073869, -83.2742425]; Comer (3921 Comer Paoli Rd.), 6-XI-2014, K. Fields (specimen) [34.0878353, -83.1133751].

DNA barcoding. Aligned COI sequences recovered in this study were compared with published sequences for *Nephila clavata* retrieved from GenBank as referenced in Su et al. (2011) and Pan and Pan (2014). Sequences from GMNHTC 12241 (GenBank Accession KP226137) and GMNHTC 12242 (Genbank Accession KP226138) were identical to each other but also included a haplotype not reported by Su et al. (2011) or Pan and Pan (2014). Sequences for *Nephila clavata* reported by Su et al. (2011) were from Yunan, China (GenBank HQ441928), Saitama, Japan (GenBank HQ441929), and Kaohsiung, Taiwan (GenBank HQ441927) and the sequence deposited by Pan and Pan (2014) was from Huaining, Anhui province, China (GenBank KJ577713). Our sequences from Georgia had a 99.9% (eValue=0) similarity to the sequences reported from Japan and China, using a Geneious megablast search. The sequence from Taiwan differed by multiple SNPs.

DISCUSSION

Hundreds of non-native species of plants and animals have been accidentally introduced into managed landscapes and natural ecosystems of North America, since the arrival of the first European explorers and colonists. Moreover, an estimated 2,000+ species of insects and arachnids have become established in North America over the past half-century, attributable to a dramatic increase in travel and international commerce (OTA, 1993). To date, approximately 60 species of non-native spiders (Araneae) have been detected in North America, with the majority originating from Europe and Asia (species list posted on http://bugguide.net/node/view/32329#Anchor_Araneae). *Nephila clavata* becomes the newest species to be added to this list of non-native spiders in North America.

Potential pathways of introduction. Accidental human transport of spiders and their egg masses in cargo containers, on plant nursery stock, and on crates and pallets, can explain and account for the presence of many European and Asian species in North America. We think this is the probable means of transport by which *N. clavata* gained entry. If accidental transport of *N. clavata* were to occur in late August to early October from countries of origin in East Asia, then the spiders' reproduction would be at its height and there would be a greater likelihood that egg masses might be deposited on structures or plant material being exported.

Source country of origin. Sequence data for the COI gene recovered from these Georgia specimens confirms our identity of *N. clavata*, by comparison with published sequences from four different Asian populations of *N. clavata*, and suggests that specimens recorded from Georgia might be more closely

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related to sources in China or Japan, than Taiwan. The Georgia specimens sequenced span the geographical range of our records and suggest that they share the same source. The Hunan and Huaining specimens are over 2,000 km distant from each other and the Saitama, Japan population is over 6000 km from Hunan, and all of these sequences are identical for about 700 base pairs of COI. The occurrence of the same unique SNP for each of the Georgia specimens however, suggests a different source population in Asia than the published sequences the Georgia specimens are most similar too. Further sequence comparisons will be necessary to confirm the country of origin or general region.

Intra- and interstate dispersal opportunities. Once a foothold is achieved, a spider's movement within northeast Georgia and adjacent states might be better explained by the major rail or road corridors across the state or possibly by the ballooning of spiderlings in the spring after egg hatch. Ballooning is a behavior by which spiders use air-borne dispersal to move between locations. Depending on mass and posture, a spider might be taken up into upper air streams (Suter, 1992). Also, its aerial movement would be dependent on convection air currents and on the drag of the silk parachute (Greenstone, Morgan, and Hultsh, 1987).

A preponderance of sightings and collection of specimens of *N. clavata* have been centered on a restricted area in and around Braselton and Hoschton, GA. One property owner in Hoschton indicated to us that the spider has been present around her home for at the past 4 years. We are not necessarily suggesting that this area represents the probable arrival point of this Asian spider, but it could be argued that the industrial and business history of the region might demonstrate it to be a possibility. The town of Braselton is a thriving business location on the I-85 business corridor, located 64 km northeast of Atlanta. As such, its location on the I-85 corridor provides excellent transportation access. It is home to many warehousing and distribution facilities that transport containerized freight from overseas.

Collections locales in Jackson and Barrow counties (n=7) are clustered on the I-85 corridor but the Madison county records (n=2) are located in a rural mixed farm landscape, not adjacent to commercial transportation corridors. The Madison county sites are ca. 50 km due west and downwind from the other Barrow and Jackson county sites. We hypothesize that these downwind sites were colonized by aerial dispersing spiderlings using the prevailing westerly winds and suggest that other populations might be found along this route.

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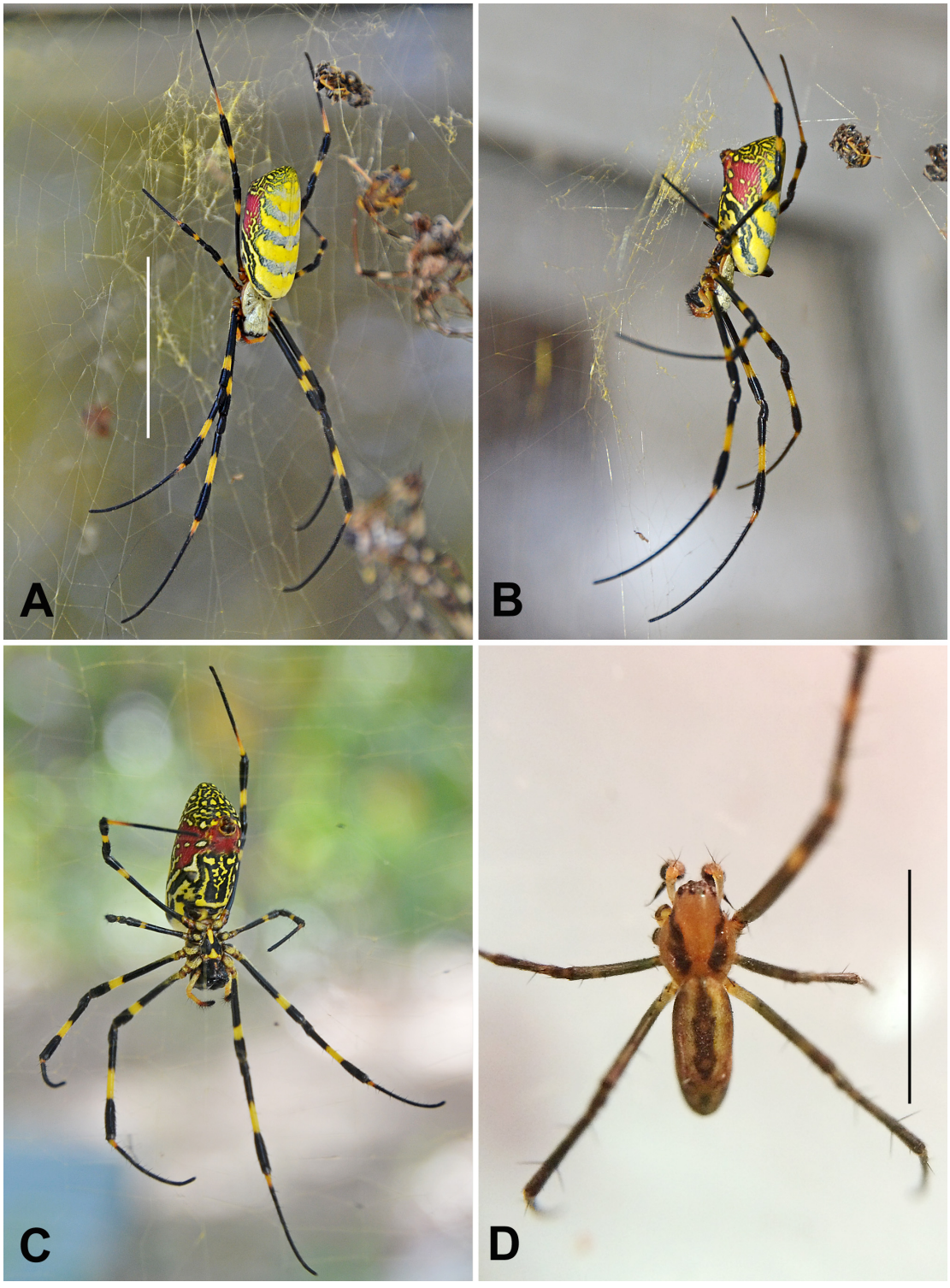

Figure 1. Photographic images of *Nephila clavata*, the Joro spider, suspended in its web in northeast Georgia, taken in October 2014. A, female, dorsal aspect; scale bar = 30 mm. B, female, lateral aspect. C, female, ventral aspect. D, male, dorsal aspect; scale bar = 5 mm. Photos A-C were taken in Hoschton, GA by Jeremy Howell; photo D was taken in Braselton, GA by B. J. Freeman.

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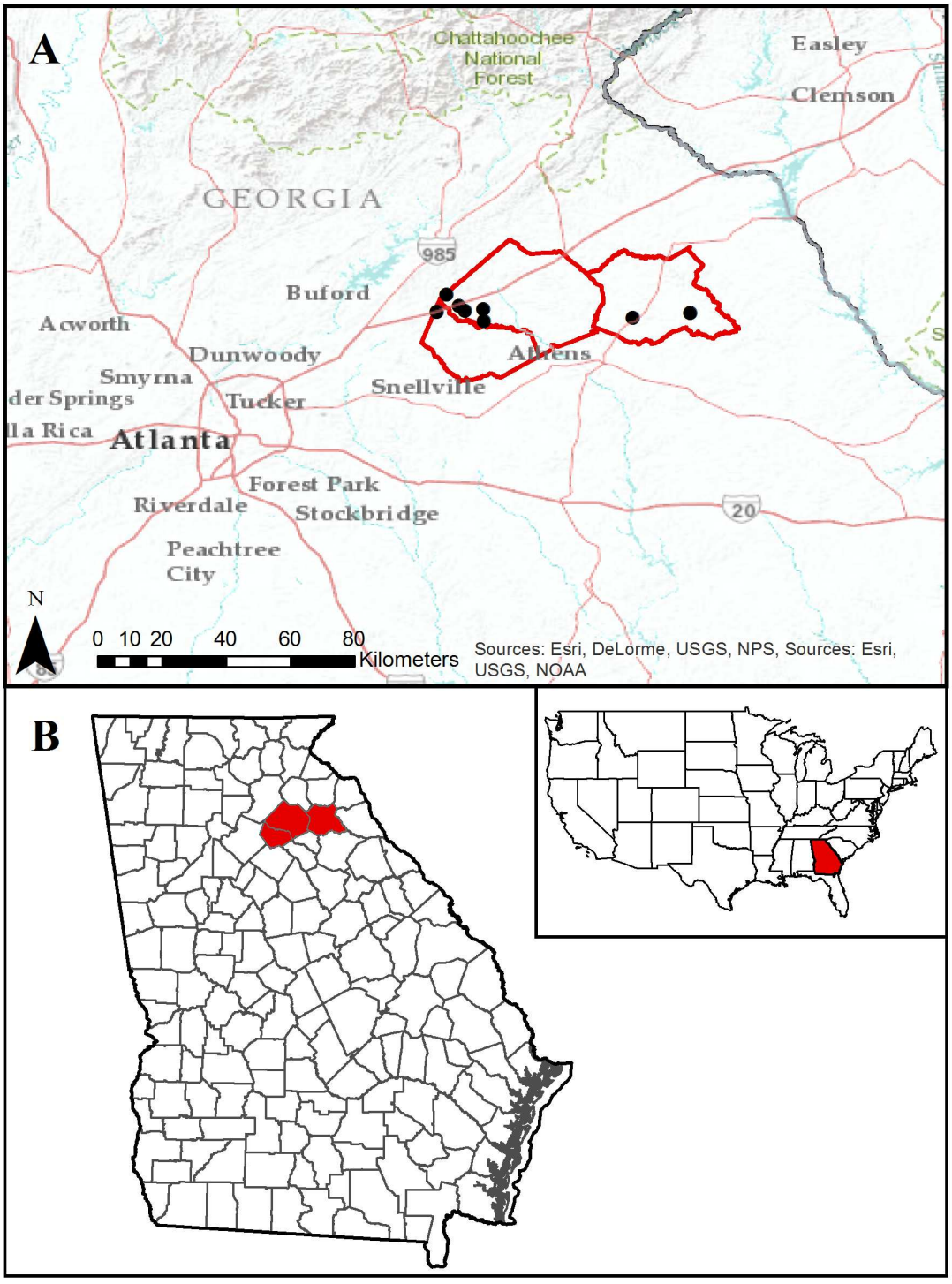

Figure 2. Map of Georgia, U.S.A. A, geocoded localities in Barrow, Jackson, and Madison counties, about 64 kilometers northeast of Atlanta, where collections and sightings of *Nephila clavata* were made. B, location of the above-mentioned counties in northeast Georgia.

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