Using DNA Barcodes to Confirm the Presence of a New Invasive Cockroach Pest in New York City

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ABSTRACT Recently, specimens of a *Periplaneta* sp. were discovered in New York, NY, that did not match the typical morphology of *Periplaneta americana* L., the ubiquitous American cockroach. Here, we used DNA barcoding and morphological identification to confirm that this newly invasive pest species was indeed *Periplaneta japonica* Karny, 1908. We discuss this recent invasion in light of known life history traits of this species, with specific predictions for its impact in the urban northeastern United States.

KEY WORDS Invasive, cockroach, pest, barcode, *Periplaneta japonica*

The probable native range of *Periplaneta* Burmeister consists of the continents of Asia and Africa (Guthrie and Tindall 1968). In the latter case, populations were presumably brought over to the Americas upon the start of European trade (e.g., slave trade and others) that began during the early 1600s (Bell and Adiyodi 1981). To date, there are at least 47 species in the genus, which was first described by Burmeister in 1838. The four species of *Periplaneta* that are established in North America are *Periplaneta americana* L., *Periplaneta fuliginosa* Serville, *Periplaneta brunnea* Burmeister, and *Periplaneta australasiae* (F.), all of which are common peridomestic cockroaches in North and South America (Hagenbuch et al. 1988, Atkinson et al. 1990). Until this present article, *Periplaneta japonica* Karny, 1908 was thought to be restricted to Japan and Asia (i.e., Japan, China, and southeastern Russia; Beccaloni 2007, Princis 1966). *P. japonica* is considered a domiciliary pest in its Japanese native and invaded Asian range, existing in residential and commercial dwellings (Tanaka 2002). *P. japonica* is found in central and northern Japan (and similar latitudes in invaded China; Xin-bei et al. 1995), and is adapted to northern climates with uni- and semivoltine ecology (Tanaka and Uemura 1996). Remarkably, their nymphs are able to survive on ice for long periods and may also be able to survive temporary freezing through their internal use of trehalose (Tanaka and Tanaka 1997, Tanaka 2002). This behavior has not been found in the current U.S. *Periplaneta* pest species, which raises questions about the potentially unique ability of *P. japonica* nymphs to overwinter outdoors in cold eastern North American climates. In China, *P. japonica* has its highest population densities in the summer from June through August, going through eight instars over a 2- to 7-month lifespan (Gengcheng 1990, Xin-bei et al. 1995).

Despite being distantly related, ectobiid and blattid pest cockroach taxa are similar in that they both contribute to human health and disease. They contribute to poor indoor air quality through the release of chemicals that are considered allergens (Arruda et al. 2001). In general, these cockroaches, which include *P. americana* and probably *P. japonica*, contribute to allergies and asthma (Baumholtz et al. 1997, Rosenstreich et al. 1997, Arruda et al. 2001, Bonnefoy et al. 2008). Pest cockroaches, including the *Periplaneta* spp., are linked with numerous medically important pathogens (Roth and Willis 1960, Baumholtz et al. 1997). For example, both *Periplaneta* spp. and *Blattella* spp. were found to be responsible for an outbreak of *Klebsiella pneumonia* (Cotton et al. 2000). One study isolated 13 species of fungi from the surface of common American cockroaches, *P. americana*, from a single hospital in Brazil (Lemos et al. 2006). Cockroaches are also significant carriers of food-borne parasites (El-Sherbini and El-Sherbini 2011, Baumholtz et al. 1997).

Here, we discuss the identification, presence, and potential impact of a new invasion of *P. japonica* in the United States.

Materials and Methods

Specimens were discovered and collected from the High Line public garden in New York, NY, in 2012. The High Line, which opened in two phases (first in 2009 and second in 2011), is an elevated 395-acre (160-ha) public park built on an old railway line located in the Meatpacking District between 10th and 11th Avenues. The park consists of naturally overgrown areas, walkways, and planted vegetation.

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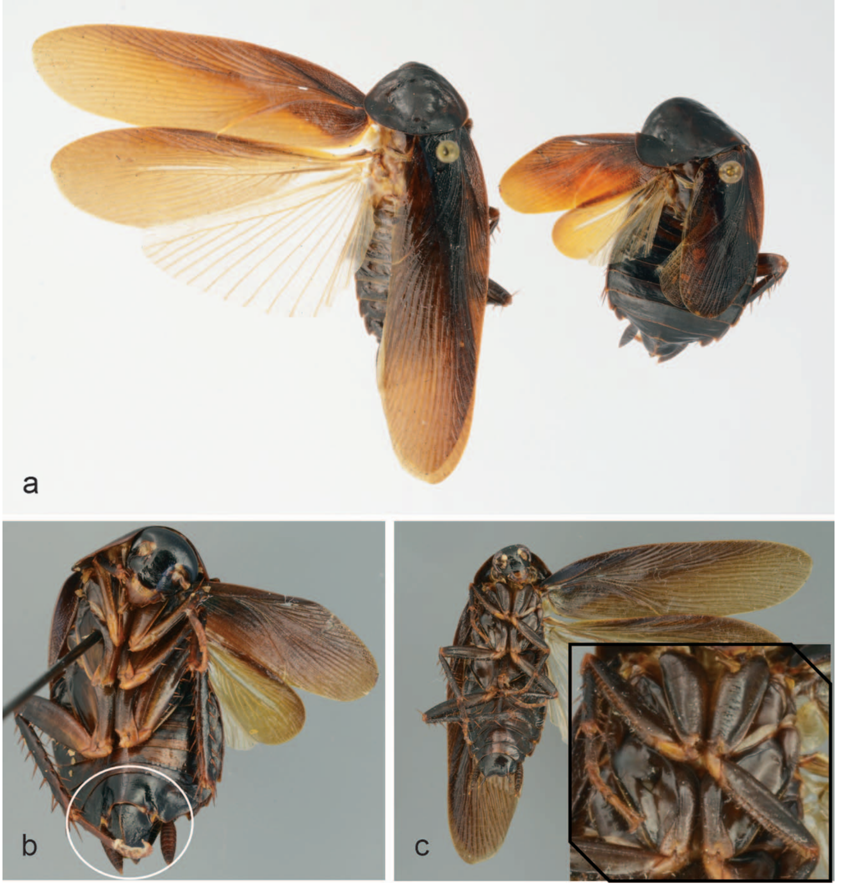

Fig. 1. (a) Shows the dorsal aspect of the male (left) and female (right) specimens; (b) shows the ventral aspect of the female with the subgenital plate highlighted; (c) shows the ventral aspect of the male and a magnified view of the forelegs. Male is 26 mm long, and female is 17 mm long.(Online figure in color.)

Morphological Identification. Roth (2003) discussed problems and issues in the identification of cockroaches when taxonomic keys are unavailable, which is the great majority of the time. Here, we did not use a key for the identification of these specimens.

Figure 1 shows dorsal and ventral aspects of the specimens treated. On examination of the specimens, the valvate subgenital plate of the female (Fig. 1b) gave a clear identification of the family Blattidae. The spination on the ventroanterior margin of the forefemur is also consistent with this identification, with the spines uniform in size from the base to the apex. The sexual dimorphism (variation in wing size) apparent in *P. japonica* is unique among other North American pest *Periplaneta* species (D. E., unpublished data). However, care is recommended, as a superficial examination using relative wing size may falsely lead to identification as the Oriental cockroach *Blatta orientalis* L.

Once this identification was ascertained, an unconfirmed identification of *Periplaneta japonica* Karny was reached through visual comparison with photographs, illustrations, and preserved specimens.

Molecular Identification. DNA was extracted from the middle legs of the two specimens morphologically identified as *P. japonica* using a Qiagen DNA extraction kit (Qiagen Operon, Alameda, CA). After extraction, samples were amplified by polymerase chain reaction (PCR). The cytochrome oxidase-1 gene fragment was chosen because it is commonly used for species identification (Hebert et al. 2003, Wiemers and Fiedler 2007, Cerutti-Pereyra et al. 2012). It was amplified by PCR using previously published LCO and HCO primers (Folmer et al. 1994). The PCR program was as follows: 96°C for 2 min, then 30 cycles of 94°C for 15s, 49°C for 25s, 72°C for 30s, 94°C for 15s, 52°C for 25s, 72°C for 30s, and a final step of 72°C for 10 min. Two different temperatures were used for the annealing step in an attempt to increase the yield of amplified DNA. Successfully amplified samples were sequenced at Macrogen (Macrogen Inc., New York, NY). Following sequencing, contigs were assembled using the Sequencher software (Gene Codes Corporation, Ann Arbor, MI). Maximum likelihood (ML) analyses were conducted on a total dataset including GenBank cockroaches using the Genetic Algorithm for Rapid Like-

Table 1. Taxa used in phylogenetic reconstruction

GenBank no.	Taxon	Reference
JX402723	*Blatta orientalis*	(Pava-Ripoll, Pearson, and Ziobro, unpublished)
KC617795	*Blatta orientalis*	(Jones et al. 2013)
KC617797	*Blatta orientalis*	–
KC617798	*Blatta orientalis*	–
AY165646	*Periplaneta americana*	(Hebert et al. 2003)
JN900479	*Periplaneta americana*	(Oshaghi, Hashemi Aghdam, and Mohtarami, unpublished)
JQ267476	*Periplaneta americana*	(Oshaghi, Hashemi Aghdam, Akbariand Shiravi, unpublished)
JQ267477	*Periplaneta americana*	–
JQ267478	*Periplaneta americana*	–
JQ267479	*Periplaneta americana*	–
JQ267480	*Periplaneta americana*	–
JQ267481	*Periplaneta americana*	–
JQ267482	*Periplaneta americana*	–
JQ267483	*Periplaneta americana*	–
JQ267484	*Periplaneta americana*	–
JQ267485	*Periplaneta americana*	–
JQ267486	*Periplaneta americana*	–
JQ267487	*Periplaneta americana*	–
JQ267488	*Periplaneta americana*	–
JQ267489	*Periplaneta americana*	–
JQ267499	*Periplaneta americana*	–
JQ350707	*Periplaneta americana*	(Cho, Suh, Kim, and Bae, unpublished)
JX402724	*Periplaneta americana*	(Pava-Ripoll, Pearson, and Ziobro, unpublished)
KC617844	*Periplaneta americana*	(Jones et al. 2013)
KC617845	*Periplaneta americana*	–
KC617846	*Periplaneta americana*	–
KF155026	*Periplaneta australasiae*	(Evangelista et al. 2013)
KF155034	*Periplaneta australasiae*	–
KF155035	*Periplaneta australasiae*	–
KF155036	*Periplaneta australasiae*	–
AM114930	*Periplaneta brunnea*	(Chaumot et al. 2012)
JQ350729	*Periplaneta fulginosa*	(Cho, Suh, Kim and Bae, unpublished)
JQ350708	*Periplaneta japonica*	–
KC407710	*Periplaneta japonica*	–
KC407711	*Periplaneta japonica*	–

lihood Inference program (GARLI; Zwickl 2006; Table 1). Before analyses, the Hasegawa–Kishino–Yano evolutionary model was chosen based on Akaike information criterion using jModeltest (Posada 2008). Analyses ran with default settings for run termination, and branch support was estimated via a 100 bootstrap pseudo-replicate analysis.

Results

The cockroaches were found at the High Line park, living in rodent bait stations, in the spring of 2012. They have also been observed in sprinkler control boxes, mulch piles, and other areas near the soil. The largest groups of individuals were seen under the boardwalk near beds of trees. Of the specimens collected, two were taken for identification and are shown in Fig. 1. The male specimen is 26 mm in length and the female is 17 mm in length.

Placement of the sequences for these unknown taxa was a positive match for identification as *P. japonica* using GARLI ML analyses (Zwickl 2006; Fig. 2). The "unknown" specimens were recovered in a monophyletic clade with other *P. japonica.*

Discussion

This is, to knowledge of the authors, the first record of this species of cockroach in the United States. Because they were collected from a public garden, we can speculate that one possible mode of introduction of this population is transportation to the United States with ornamental plants, although several other scenarios are possible. Given the special ability of *P. japonica* to withstand freezing temperatures (Tanaka and Tanaka 1997, Tanaka 2002), the potential of this species to have a successful invasion is greatly increased, as they may be able to withstand the frigid winters that would likely kill populations of other potential pest cockroaches (e.g., *P. australasiae*). This may also mean that populations of *P. japonica* are not only limited to a distribution within structures because they can live without anthropogenic heat sources. Ecological dynamics between *P. japonica* and other cockroach species already present in dwellings (e.g., the American cockroach) are unknown, but it is possible that the difference in the temperature tolerance niche axis is enough to allow them to coexist in close proximity without competitively excluding one another. If such is the case, *P. japonica* would likely be a nuisance. However, they may not contribute as greatly to degradation of indoor air quality and the transmission of surface pathogens as other species that are more restricted to the indoors.

Invasions of nonnative animals are a concern, and in the event of cockroach invasions, domestic colonization and the subsequent degeneration of indoor quality of life are at risk. Given another recent report of a

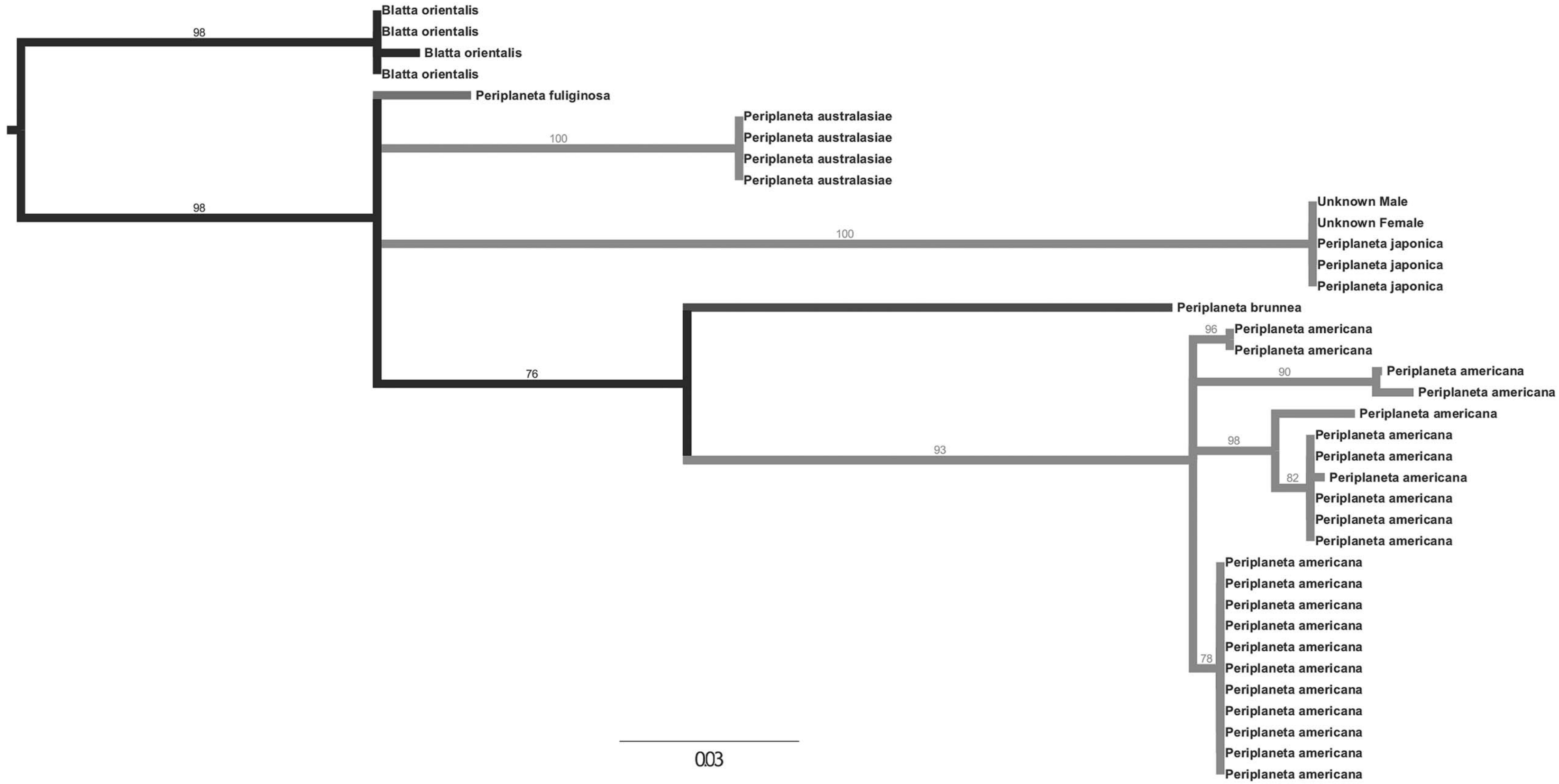

Fig. 2. Consensus tree of 100 bootstrap replicates in GARLI ML analyses of *Periplaneta* from GenBank (Table 1) and the two unknown *P. japonica* specimens.

newly introduced cockroach (Peterson and Cobb 2009) and the potentiality of their impact on human health and the economy, these populations should be monitored and researched more thoroughly.

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