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Rapid Communication

First record of a New World ant species (Hymenoptera: Formicidae), *Strumigenys eggersi* Emery, 1890 in the Old World

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Abstract

The Neotropical ant *Strumigenys eggersi* is recorded for the first time in the Old World, specifically from Singapore. Six queens and 10 workers in total were collected from Malaise trap (4 queens) and leaf litter samples (2 queens, 10 workers) respectively, on the National University of Singapore (NUS) campus. DNA barcodes (COI, 313 bp) amplified from these specimens closely matched those of *S. eggersi* on NCBI GenBank and BOLD databases, in agreement with morphology. Implications of this discovery are briefly discussed.

Key words: Neotropical ant, invasive tramp, Myrmicinae, new discovery

Introduction

The Neotropical ant *Strumigenys eggersi* Emery, 1890 (Hymenoptera: Formicidae) was originally described from St. Thomas, West Indies. It was classified as a member of the *Pyramica gundlachi* species group (Bolton 2000) (currently placed in *Strumigenys*), the members of which are morphologically distinct from other *Strumigenys* species in terms of traits such as: more remote mandibular insertions, reduced teeth on apical fork of mandible, reduced spongiform appendages of petiole and postpetiole, and general overall habitus particularly head shape (Brown 1959). Although quite similar to *S. gundlachi*, *S. eggersi* may be distinguished from the latter by its relatively smaller size and shorter mandibles (Brown 1959; Bolton 2000).

The natural range of *S. eggersi* has been postulated to span from Trinidad and the Guianas to southeastern Brazil and Amazonian Bolivia (Brown 1962). It is also known to be widespread, possibly by anthropogenic introduction through nursery stock transport and commerce, throughout the West Indies, southern Florida and southern Mexico (Brown 1959; Brown 1962). In more recent times, the species has been reported from various scattered localities in the Americas, including oceanic islands such as American Samoa (Peck and Banko 2015) and the Galapagos Islands (Herrera et al. 2014), also the island state of Barbados (Wetterer et al. 2016) and the republic of Ecuador (Salazar and Donoso 2013). It is now deemed an invasive tramp in the United States of America (USA), pervasive specifically throughout the state of Florida (Deyrup and Trager 1984; Deyrup et al. 1988, Deyrup et al. 2000). The current known distribution of *S. eggersi* in the New World is thus established from Florida (USA), all the way south to Santa Fe (Argentina), with isolate populations in American Samoa and the Galapagos (Janicki et al. 2016; http://www.antmaps.org).

Methods

Strumigenys eggersi alates were collected from Malaise trap samples as part of a 6-month campus-wide insect survey conducted by the Lee Kong Chian Natural History Museum (LKCNHM) in the National University of Singapore (NUS). Four malaise traps

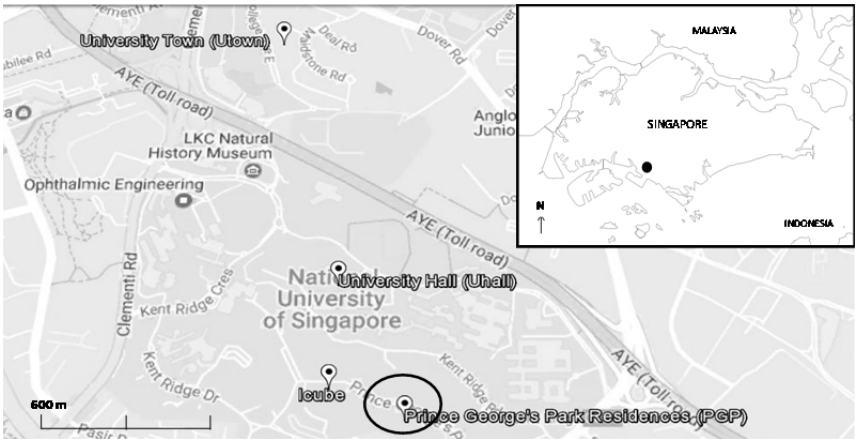

Figure 1. Map showing 4 sites in the National University of Singapore (NUS) campus sampled in the insect survey. Site where *Strumigenys eggersi* was found is encircled. Inset: Map of Singapore; black spot indicates the location of NUS.

were set up at four different locations across the NUS campus from April to September 2016 (Figure 1). Insects sampled in each trap were collected every week, after which they were presorted into higher taxonomic groups. Leaf litter at each site was also collected and processed using the Winkler apparatus, and ant samples were sorted to species based on morphological characters. Specimens were then barcoded using direct PCR amplification (Wong et al. 2014), and Next Generation Sequencing (NGS) procedures adapted largely from Meier et al. (2016).

DNA barcoding

A 313 bp fragment of cytochrome oxidase I [COI; m1COIintF: 5'-GGWACWGGWTGAACWGTWT AYCCYCC-3' (Leray et al. 2013) and jgHCO2198: 5'-TAIACYTCIGGRTGICCRAARAAYCA-3' (Geller et al. 2013)/ 5'- TAAACYTCAGGRTGCCCRAAR AAYCA-3' (Meier et al. 2016)] was amplified using labelled forward and reverse primers. Each label was 9 bp long, differing from other labels by more than 4 bp; labels were generated using the online freeware "Barcode Generator" (http://comailab.genomecenter.ucdavis.edu/index.php/Barcode_generator). We used an assortment of different combinations from 250 available pairs of labelled primers; each specimen barcode was amplified with a uniquely-labelled primer combination. The number of pairs of labelled primers required depended on number of libraries going into each MiSeq run, i.e. with 3 libraries in one run, about 40 primers pairs are needed. PCR mixtures of 20 ul reaction volume each were prepared (2 ul of 10x BioReady rTaq 10x Reaction Buffer, 1.5 ul of 2.5 mM dNTP mix, 0.2 ul of BioReady rTaq DNA polymerase, 2 ul each of 5 uM forward and reverse primers, 2 ul of 1 mg/ml Bovine Serum Albumin, RNase/DNase-free sterile water), and cycling conditions were as follows: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 47 °C for 2 min , and extension at 72 °C for 1 min, thereafter a final extension at 72 °C for 5 min.

Amplified PCR products were combined and cleaned in a maximum of 100-ul aliquots using SureClean (Bioline Inc., London, UK); cleaned amplicon products were re-eluted in RNase/DNase-free water. Next Generation Sequencing (NGS) libraries were prepared with the combined PCR products using the TruSeq Nano DNA Library Preparation kit and then sequenced on an Illumina MiSeq 2x300 bp platform. Paired-end (PE) read data (*.fastq*) were assembled using PEAR (Zhang et al. 2014).

Following routine contamination and quality checks, filtered barcode sequences were aligned and clustered according to uncorrected *p*-distances (Meier et al. 2008; Srivathsan and Meier 2012) across a range of different percentage thresholds (0–9%). Cluster splitting and/or merging events amongst individual sequences were visualized using a custom-designed software – *obj_clust* v 0.1.2 (Srithvathsan A, unpublished; an implementation of objective clustering as described in Meier et al. 2006). Clustering thresholds between 3–4% for short COI barcodes commonly reflect actual species delimitation amongst arthropods (Hebert et al. 2003a, 2003b), therefore for downstream analyses, clusters obtained at 4% threshold were used.

Identity matches for representative barcodes from each 4% cluster were searched for on the online nucleotide databases GenBank (NCBI) (Clark et al. 2016) and the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007), using default parameters of the online version of NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al.

1990). Only database matches at 100% query cover and ≥ 96% identity were interpreted as accurate barcode identities. Representative specimens per cluster were dry mounted and examined under high magnification using a Leica M80 stereo microscope. Both mounted and wet voucher specimens are deposited in the collections at Lee Kong Chian Natural History Museum.

Results

A total of 6 queens and 10 workers were collected from Malaise traps (4 alate queens) and leaf litter samples (2 dealate queens, 10 workers) respectively. These specimens were found from only one out of four sites on the campus – Prince George's Park Residences (Figure 1).

DNA analysis

COI barcodes (313 bp) obtained from the four ant queens collected using Malaise traps were completely identical (0% pairwise distances), and clustered together as a single haplotype. BLAST searches on GenBank (NCBI) did not find any hits matching our criteria for species identity. In contrast, all four barcode sequences had a 100% match with *Pyramica eggersi* (note: *Pyramica* is now synonymized with *Strumigenys*) on the BOLD barcode database (BIN URI – BOLD: AA10533); this barcode was obtained from only one worker collected from Florida, USA (SequenceID: ASANA523-06). However, barcodes for *S. eggersi* from our study were matched at a lower percentage, i.e. 94.1%, to 3 barcoded *S.eggersi* individuals collected from the Neotropics, namely 2 countries in Central America (BIN URI – BOLD: AA10534) – Nicaragua (alt. 370 m) and Costa Rica (alt. 295 m). Another two barcodes belonging to 2 individuals each from Mexico (alt. 520 m) and Honduras (alt. 1140 m), were matched to the local specimen haplotype at even lower percentages – 93.8% and 93.53% respectively. COI barcode sequences from the queens have been uploaded onto GenBank (NCBI), and allocated the following accession numbers: MF134436-134439.

Systematics

Strumigenys eggersi Emery, 1890

(Figures 2A–C (worker); 3A–C (queen))

Strumigenys eggersi Emery, 1890: 69, pl. 7, fig. 9 (w.q.). Combination in *Pyramica*: Brown 1948: 110; Bolton 1999: 1673; Bolton 2000: 184. *Strumigenys eggersi* Emery: Baroni Urbani and De Andrade 2007: 119.

Types. *Strumigenys eggersi* Emery, 1890; type locality: ANTILLES, St. Thomas, West Indies. 2 syntype workers (coll. G. Mayr; NHMW) examined.

The abbreviations used for measurements and indices are as follows:

EL: Maximum eye length measured along its maximum diameter.

EW: Maximum eye width measured in full face view.

TL: Total body length, roughly measured in lateral view from the anterior margin of the head to the apical tip of the gaster for outstretched specimens.

HL: Maximum head length in full-face view, measured in a straight line, from the anterior clypeal margin to the midpoint of a straight line drawn across the occipital margin of the head.

HW: Maximum head width behind the eyes, measured in full-face view.

SL: Maximum length of the scape measured in a straight line, excluding the basal constriction and condylar bulb.

ML: Weber's mesosomal length, measured as the diagonal length of the mesosoma in profile, from the anteriormost point at which the pronotum meets the cervical shield to the posterior basal angle of the metapleuron.

MandL: Straight line length of the mandible at full closure, measured from mandibular apex to the anterior clypeal margin, in full face view.

PronW: Maximum width of the pronotum in dorsal view, ignoring projecting spines, tubercles or other circular prominences at the humeral angles.

CI: Cephalic index, HW/HL × 100

REL: Relative eye length, EL/HW × 100

MI: Mandibular index, MandL/HL × 100

SI: Scape index, SL/HW × 100

Worker Measurements

Syntype workers (n = 2): TL 1.79–1.87 mm; HL 0.44 mm; HW 0.34–0.37 mm; EL 0.04 mm; EW 0.024–0.03 mm; MandL 0.25–0.26 mm; ML 0.48 mm; PronW 0.22–0.23 mm; SL 0.18–0.2 mm; CI 77.27–84.09; REL 10.81–11.76; MI 56.82–59.09; SI 52.94–54.05.

Central American workers (Costa Rica, n = 2): TL 1.76–1.8 mm; HL 0.41–0.42 mm; HW 0.34–0.36 mm; EL 0.04–0.044 mm; EW 0.036 mm; MandL 0.24–0.26 mm; ML 0.44–0.45 mm; PronW 0.23–0.24; SL 0.18–0.2 mm; CI 82.93–85.71; REL 11.76–12.22; MI 57.14–63.41; SI 52.94–55.56.

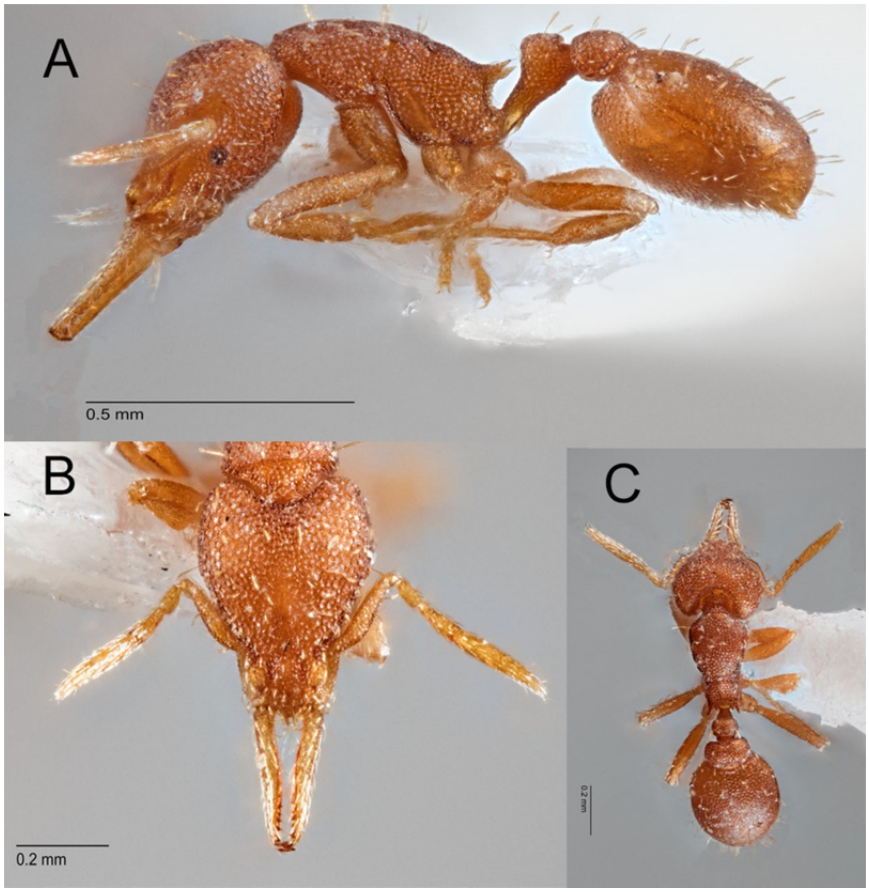

Figure 2. Worker of *Strumigenys eggersi* collected from Singapore (ZRC_HYM0000608). A: Profile view; B: full face view; C: dorsal view. Scale bars: 0.5mm profile view, 0.2 mm full face and dorsal views. Photo credit: G.W. Yong and W. Wang.

Non-type workers collected from Singapore (n = 10): TL 1.74–1.88 mm; HL 0.41–0.44 mm; HW 0.35–0.38 mm; EL 0.036–0.04 mm; EW 0.024–0.06 mm; MandL 0.25–0.26 mm; ML 0.42–0.46 mm; PronW 0.23–0.24 mm; SL 0.19–0.2 mm; CI 83.33–86.36; REL 9.73–11.43; MI 59.09–61.9; SI 52.63–57.14.

Description of Worker (based on Singapore specimens, n = 10)

Head in full face view subcordate, with occipital margin broadly and moderately concave. Antennal scrobe large and deep, extending across more than half of head in lateral view. Eye moderately small, composed of 9–11 ommatidia, located medially on lateral face of head close to ventral margin of antennal groove. In full face view, clypeus triangular, frontal lobes laterally expanded and covering antennal sockets. Antenna comprising 6 segments; scape short, when lain back not reaching posterior margin of head; second funicular (first flagellar) segment much shorter than most of remaining segments; third only slightly longer than second. Mandibles slender and almost linear, gradually tapered towards apex, with inner margins weakly convex, leaving a continuous central space even upon their full closure; mandible with 2 acute apical teeth, flanked by 6 small pre-apical denticles distributed along distal 1/2–1/3 length of masticatory margin.

In dorsal view, anterior margin of pronotum strongly convex and lamellate; humeral angles rounded. Mesosoma in dorsal view near-goblet in shape. Demarcation of pronotum and mesonotum indistinct, but metanotal groove deep. Dorsolateral margin of mesosoma lamellate, including propodeal spines. In profile view, dorsal margin of mesosoma weakly convex and continuous. Propodeal declivity concave; propodeal spines triangular and acute apically, pointing posteriorly. Petiole with elongate peduncle; in profile, petiolar node rounded dorsally, its articulation with helcium defined by an angular junction; in dorsal view sub-rectangular in shape with four corners gently rounded; anterodorsal margin weakly convex. Postpetiole slightly shorter than petiole, globular in dorsal view, much broader than petiole, with anterior margin broadly concave; in profile both dorsal and ventral margins rounded.

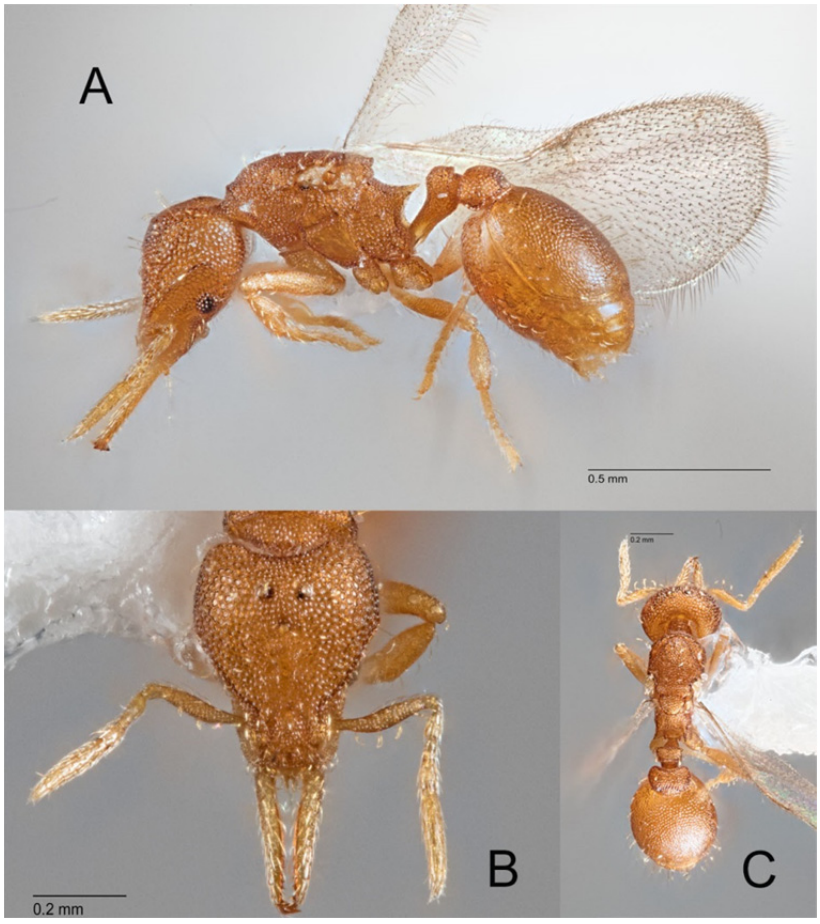

Figure 3. Queen (alate) of *Strumigenys eggersi* collected from Singapore (ZRC_BDP0045491). A: Profile view; B: full face view; C: dorsal view. Scale bars: 0.5 mm profile view, 0.2 mm full face and dorsal views**.** Photo credit: G. W. Yong and W. Wang.

Subpetiolar and sub-postpetiolar processes reduced to low lamellae. Spongiform appendage on waist vestigial to almost absent. First gastral tergite much larger than the rest, in dorsal view elliptical in shape.

Surfaces of head, scapes and clypeus densely reticulate-punctate with interspaces punctured but shining. Mandibular surface striated and shining, basal 1/3 with dense punctures that become more scattered towards apex. Mesosoma extensively densely reticulate with shining interspaces; katepisternum of mesopleuron smooth and shining in contrast with rest of mesosoma. Petiole and postpetiole also reticulate-punctate but still shining. Surface of first gastral tergite areolate-punctate basally, becoming more shagreened posteriorly but still weakly shining. Anterolateral sides of first gastral tergite with a few short carinae.

Dorsal surface of head covered with abundant suberect clavate hairs; mandibular surface also covered with numerous short appressed hairs, mostly simple. One pair of short, erect, slightly clavate hairs present on pronotal humeri; a pair of similar standing hairs flanking dorsolateral part of propodeum anteriorly. Two other pairs of slightly clavate standing hairs present on dorsolateral margins of mesonotum. Dorsal surfaces of petiole, postpetiole and gaster with scattered, but orderly arranged short standing hairs that are weakly clavate. Ventral surface of gaster with short, simple, fine standing hairs concentrated mostly in its posterior half.

Queen Measurements

Non-type dealate queens from Central America (Costa Rica, Nicaguara, n = 2): TL 2.06–2.08 mm; HL 0.45 mm; HW 0.38–0.4 mm; EL 0.07–0.08 mm; EW 0.05 mm; MandL 0.29 mm; ML 0.52 mm; PronW 0.26–0.29 mm; SL 0.21–0.22 mm; CI 84.44–88.89 ; REL 18.42–20; MI 64.44; SI 52.5–55.26.

Non-type queens from Singapore (n = 6, 4 alate, 2 dealate): TL 2.01–2.04 mm; HL 0.45 mm; HW 0.38–0.4 mm; EL 0.064–0.08 mm; EW 0.04–0.05 mm; MandL 0.27–0.28 mm; ML 0.5–0.52 mm; PronW 0.26–0.28 mm; SL 0.22–0.23 mm; CI 84.44–88.89; REL 16–20.51; MI 60–62.22; SI 52.5–52.63.

Description of queen (based on Singapore specimens, n=6)

Structural features mostly close to those of workers, except for characters exclusive to gynes. Eye larger than in workers, with 23–24 ommatidia, located medially on lateral face of head just below ventral margin of antennal groove. With mesosoma in dorsal view, anterior margin of pronotum weakly convex and carinate; humeral angle relatively rounded. Mesosoma in dorsal view near-trapezoidal in shape, in profile with weakly and evenly convex outline; mesoscutum slightly longer than wide; metanotum with almost vertical posterior face that is distinctly demarcated from dorsal face. Propodeal spine triangular, acute apically and pointing posteriad; its base almost as broad as petiolar height, broader and more robust than in workers. Petiole with long peduncle; in profile its node with anterodorsal and posterodorsal margins angulate, in dorsal view much broader than long; posterior face laterally and dorsally margined with lamellae; subpetiolar process reduced to weak carina. Postpetiolar node much larger than petiolar node, rounded, seen from above distinctly broader than long.

Surface sculpture and pilosity largely similar to those of workers. Gastral sculpture coarse and prominent compared to that of workers. One pair of short, erect, clavate hairs present on pronotal humeri, followed by sparse paired, short and clavate standing hairs roughly arranged along dorsolateral margins of mesosoma.

Sampled material examined. SINGAPORE**:** 4 alate queens, National University of Singapore, Prince George's Park Residences, 1º17′32.6″N; 103º46′43.3″E, 27 May 2015; 10 Jun 2015; 24 Jun 2015, malaise trap samples (*M.S. Foo*) (NUS0033/41/49) (ZRC_BDP0044391/45491/46920/46922); 2 dealate queens and 10 workers, same locality as above, 5 April 2017, leaf litter Winkler method (*W. Wang*) (ZRC_HYM_0000608).

Additional non-type material examined. COSTA RICA: 2 workers, 1 dealate queen, Prov. Gua F. La Pacifica, 40m, 10º27′N; 85º08′W, 16–17 July 1985, dry forest litter sample (*J. Longino*) (530-5) (JTLC, INBIO CRI001 283813). GUADELOUPE, Basse Terre: 1 dealate queen, Sentier Houélmont, 15.98135N; 61.70947 W ± 50 m, 105 m, 18 May 2012, lowland deciduous forest, ex sifted leaf litter (*R.S. Anderson*) (RSA2012-121) (JTLC, CASENT0627391); 1 dealate queen, Pigeon, Sous-Le-Vent, 16.15042 N; 61.76414W ± 50m, 194 m, 12 May 2012, dry trop. decid. forest, ex. sifted leaf litter (*R.S. Anderson*) (RSA2012-100) (JTLC, CASENT0630643). MEXICO, Chiapas: 1 dealate queen, 12 km NW Flor de Café, 16º08′N; 91º16′W, 520 m, 22 July 2007 (*J. Longino*) (6103-s) (JTLC, JTLC000010117). NICARAGUA, Raan: 1 dealate queen, PN Cerro Saslaya, 13.77398N; 84.98530W ± 20 m, 470 m, 8 May 2011, disturbed tropical wet forest, ex. sifted leaf litter (*LLMA*) (Wm-D-02-1-04) (JTLC, CASENT0628850). VENEZUELA, Aragua: 1 dealate queen, Ocumares de la Costa, 10.46009N; 67.77643W, 70 m, 13 Aug 2008, second growth dry forest, ex. sifted leaf litter (*J. Longino*) (6449-s) (JTLC, JTLC000015057).

Remarks

The varied divergences of COI barcodes between specimens morphologically identified as *Strumigenys eggersi* collected from Singapore, Florida and the Americas suggest the existence of a species complex. At this stage, however, we are unable to infer anything conclusive based on the small number of individuals barcoded from the species' native range, which may not accurately represent actual genetic variation across native populations in general.

Morphologically, specimens from Singapore are almost identical to those from the New World. Morphological measurements were similar amongst syntype workers and non-type workers collected from both Central America and Singapore; most metrics measured for workers from both regions were within ranges as stated in Bolton (2000), except for scape length (SL), and correspondingly, scape index (SI). Specimens measured in this study all seemed to have slightly shorter scapes, under the range indicated in Bolton (2000). This discrepancy may suggest the existence of a species complex, but it may also be because our small sample size (n = 10) does not represent the true range of SL in the Singapore population. There were also slight differences between queens from different regions—queens collected from Singapore seemed slightly smaller in general as compared to those from Central America. Our sample size in this study is too small to capture actual size variation among queens from populations in both regions, therefore we do not rule out the possibility that queen size differences may simply be an artefact of insufficient sampling.

Strumigenys eggersi is known to be resilient in the New World, being adapted to disturbed forests and hot/dry conditions; their habitat preferences do not appear restrictive, and they have been found to thrive in varied habitats (Deyrup 2016). In Florida, these ants are commonly found in both moist and dry habitats, including natural woods and man-made gardens, where they often nest in hollow twigs or leaf litter (Deyrup et al. 2000; Deyrup 2016). Previously barcoded individuals on BOLD were also collected

from leaf litter in lowland dry forest (Costa Rica), and even at higher elevations (Honduras, alt. 1140 m). It is therefore not surprising that *S. eggersi* is able to thrive in a small patch of disturbed vegetation in a wholly urban matrix, though its precise introduction to this region remains a mystery. In Singapore the species should have established itself since both workers and winged queens have been collected. However, they were sampled in a site with relatively restricted access, thus actual colonies have not yet been found. While undeniably exotic, the effects of *S. eggersi* on native ecosystems have not been formally established, and thus it is not currently considered to be a harmful invasive. Nevertheless, periodic monitoring of the species should be conducted in future as a precaution.

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