2013

DNA Barcode of Thief Ant Complex (Hymenoptera: Formicidae)

Ralph B. Narain
University of Nebraska-Lincoln

Shripat T. Kamble
Universitiy of Nebraska--Lincoln, skamble1@unl.edu

Thomas O. Powers
University of Nebraska-Lincoln, tpowers1@unl.edu

Timothy S. Harris
University of Nebraska-Lincoln

DNA Barcode of Thief Ant Complex (Hymenoptera: Formicidae)[1]

Ralph B. Narain, Shripat T. Kamble[2] Thomas O. Powers[3] and Timothy S. Harris[3]

Department of Entomology, University of Nebraska, Lincoln, Nebraska 68583 USA

Abstract   The thief ant, *Solenopsis molesta* (Say), a common nuisance species found through-out the United States is genetically related to red imported fire ants, *S. invicta* Buren. Therefore, its identification at the molecular level is very important. The deoxyribonucleic acid (DNA) barcoding, a recent technique was used to identify thief ant complex at species and subspecies levels using a short DNA sequence from the cytochrome oxidase subunit 1 (COI) mitochondrial region. The DNA from thief ants collected from 9 states was extracted using Qiagen's Gentra PUREGENE® DNA Isolation Kit. The polymerase chain reactions (PCR) were run on the extracted DNA to amplify partial sequence of COI using primers Lep-F1 (forward) and Lep-R1 (reverse). The re-sulting DNA products were concentrated, purified and sequenced. The 600 bp sequences of the COI generated were submitted to GenBank that issued accessions numbers from HM179641 to HM179653. The sequences associated with these accession numbers were used as DNA bar-codes for distinguishing species and subspecies. Based on this molecular analysis, thief ants collected from New York, Indiana and 1 location in Nebraska were separated in 1 group as *S. molesta validiuscula* (Emery) and another with ants from Louisiana identified as *S. carolinensis* (Forel). The third group was comprised of ants from South Dakota, Washington, New Jersey, Ten-nessee, Kansas and 2 other locations in Nebraska was identified as *S. molesta molesta* (Say).

Key Words   thief ants, *Solenopsis* sp., molecular genetics, DNA barcode

Ants are one of the leading causes for complaints to pest management profession-als (PMPs) from homeowners. The PMPs generated approx. US $1.7 billion annually to manage ant populations (Field et al. 2007). The thief ants (*Solenopsis molesta* Say) are included within the group of ants known as nuisance pests (Bennett et al. 2005, Klotz et al. 2008). Because thief ants are genetically related to red imported fire ants, *S. invicta* Buren, it is critical to identify these ants accurately at species level. DNA barcoding, a relatively new taxonomic approach, uses a short sequence from the mitochondrial DNA (mDNA) region as a molecular diagnostic tool for identification of species (Hebert et al. 2003). DNA barcoding sequences a section (600 bp to 650 bp) of the cytochrome oxidase subunit 1 (COI). This is relatively short compared with the mDNA genome (>16,000 bp) (Hajibabaei et al. 2007). The mDNA region suggested for the use in DNA barcoding is highly conserved and relatively easy to isolate and sequence. Several researchers have reported that COI sequence variability is low and closely related to species difference, making it possible to confidently identify species and resolve most species-level differences (Hebert et al. 2004, Rohfritsch and Borsa

2005, Ward et al. 2005, Hajibabaei et al. 2006, Hye et al. 2006, Tavares and Baker 2008).

DNA barcoding offers a standardized method for identifying species using a short mDNA sequence from the COI gene to provide a 'barcode'. DNA barcoding popularized by Hebert et al. (2003) has since gained acceptance, leading to public databases of DNA barcodes, such as the 'Barcode of Life' and 'GenBank', where the mitochondrial COI gene sequences ('barcodes') for species are stored (Hebert et al. 2003). These databases provided a central location where gene sequences for identifying species can be easily and quickly accessed.

The use of mDNA for barcoding and identification is not foolproof. DNA barcoding relies on the low levels of mDNA sequence variation within species as compared with between species. The presence of symbionts such as *Wolbachia* can disrupt this pattern by contributing mDNA sequence to their host (Hurst and Jiggins 2005, Whitworth et al. 2007). Additionally, because mDNA are maternally inherited markers, it would be unreliable if male and female histories differ in a species. Also, with conserved primers, there is the possibility of nuclear genome integrations into mDNA sequence, confounding the potential to clearly identify species. The presence of pseudogenes in mDNA and its inconsistent evolutionary rate among lineages are also disadvantageous in relying on COI as the sole marker for taxonomic identification (Chu et al. 2009).

The advantages of DNA barcoding are far greater than the disadvantages previously described. The use of DNA barcoding for species identification and population genetics is important as numerous cryptic species are misidentified. Because the DNA sequences of a species are unique, the DNA barcode developed for any species could be used to separate cryptic species (Hebert et al. 2004, Smith et al. 2006, 2007). Most cryptic species are, as Bickford et al. (2007) described, in 'morphological stasis' – limited or no changes due to selection, adaptation and/or environmental condition. Hajibabaei et al. (2006) used COI DNA barcodes to differentiate among lepidopteran families from Costa Rica and found that 97.9% (of 521) species have distinctive COI barcodes.

DNA barcoding also may be used for the early detection of invasive species and their spread such as the big-headed ant, *Pheidole megacephala* F., a pest ant registered in the list of the '100 of the world's worst invasive alien species' (Fournier et al. 2008). This technique will help with faster, more accurate species identification which could accelerate implementation of proper control methods and, thus, reduce their geographic movement. DNA barcoding can identify a species throughout its entire life cycle, whereas morphological identification of a species is based mostly on adult features. Numerous researchers have used this method to identify alien, cryptic or invasive species in entomology, botany, ornithology, ichthyology, etc.; for example, Chown et al. (2008) with lepidopteran species in Marion Island, South Africa; Hebert et al. (2004) with neotropical skipper butterfly; Lahaye et al. (2008) with plant biodiversity at 2 hotspots (southern Africa and Mesoamerica); Kerr et al. (2007) with North American birds; Ward et al. (2005) with Australian fishes, etc.

Accurate identification of the thief ants relied heavily of the morphological features of the queen of each species. Because the queens are not easily available, molecular technique using the abundant worker caste could be used for species identification. The objective of this research was to develop DNA barcodes for identifying the thief ants within the *S. molesta* complex using workers for a fast and reliable tool to identify thief ants at species and subspecies levels.

Materials and Methods

Ant collection. Thief ants, *S. molesta,* were collected from 3 locations in Lancaster Co., Nebraska and other states including: Indiana, Kansas, Louisiana, New Jersey, New York, South Dakota, Tennessee and Washington (Table 1). Thief ants in Nebraska were collected using the techniques described by Husen et al. (2008). All thief ant specimens were preserved in 95% ethyl alcohol and stored at -20°C in VWR freezer (VWR, West Chester, PA) for DNA extraction, COI amplification and sequencing.

DNA extraction and isolation. Thief ant workers stored in 95% ethyl alcohol at -20°C were removed, and the ethanol was allowed to dry. DNA was extracted from ants using PUREGENE® DNA Isolation Kit (Invitrogen, Carlsbad, CA) and tissue method modified from PUREGENE® DNA Isolation manual included in the kit. Standard primers (Smith et al. 2007) *(Forward Primer >LepF1 ATTCAACCAATCATAAAGATATTGG; Reverse primer >LepR1 TAAACTTCTGGATGTCCAAAAAATCA)* (Invitrogen, Carlsbad, CA) were used to amplify and sequence the mitochondrial COI from thief ants. Additional solutions and reagents required for DNA extraction and PCR amplification were prepared according to protocols of Sambrook et al. (1989). After completion of the amplification process, 5.0 µL PCR product was loaded into 1.0% agarose gel in 0.5x TBE, stained with 0.1% ethidium bromide, electrophoresed at 100 V for approx. 1 h. The gel was viewed and photographed (Fig. 1) on a Bio-Rad Gel Doc System (Bio-Rad, Hercules, CA). The DNA, once rehydrated, was stored at 4.0°C until PCR amplification was completed. The concentration of the extracted DNA was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA), and an equivalent of 80 - 100 ng/µl was used as template for the PCR reaction.

PCR amplification program and DNA sequencing. Polymerase chain reactions (PCR) amplification program and DNA sequencing were performed according to

Table 1. Thief ants collected from various states were used for species identification.

State	Zip Code	Latitude	Longitude	Collector
Louisiana	71051	32.3309	−93.4801	L. Hooper-Bui
Louisiana	70714	30.5922	−91.1161	L. Hooper-Bui
South Dakota	57701	44.1454	−103.1510	R. Narain
Tennessee	37721	36.1259	−83.8261	K. Vail
Nebraska	68516	40.7369	−96.6531	R. Narain
New Jersey	08901	40.4879	−74.4467	C. Wang
Kansas	66503	39.2327	−96.6852	S. Dobesh
Washington	99224	47.6733	−117.5328	L. Hensen
Nebraska	68505	40.8247	−96.6157	R. Narain
New York	11741	40.7942	−73.0700	S. Narain
Nebraska	68583	40.8002	−96.6667	R. Narain
Indiana	47907	40.4253	−86.9155	G. Buczkowski
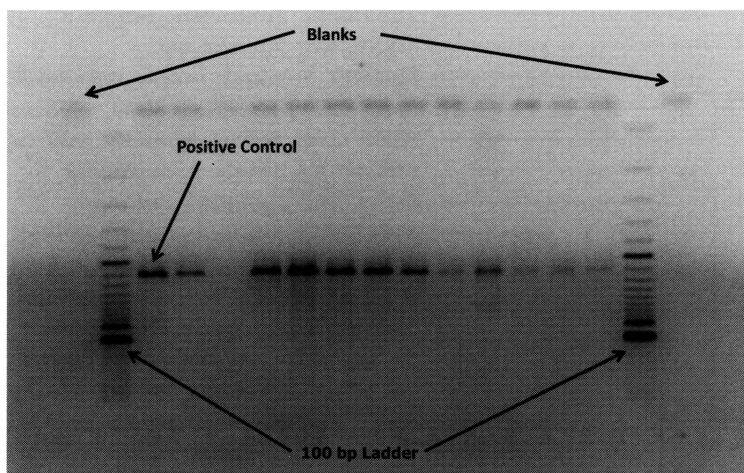
Fig. 1. Image of 1% agarose gel showing some successful and unsuccessful PCR amplification of COI DNA from thief ants, primer dimers shown as a band at the top of the gel.

protocol of Narain et al. (2012). Additional sequences of *S. invicta* used for comparison were downloaded from GeneBank, accession numbers EU677835, JN703421, JN703423 and JN703425.

Results and Discussion

Species genetic identification. Phylogenetic tree (Fig. 2) analysis using programs at www.phylogeny.fr (Dereeper et al. 2008) separates the COI sequences collected into 3 groups of thief ants previously identified (Narain et al. 2012). Included in the Neighbor Joining tree are *S. invicta* COI sequences, which were used to compare its relationship to that of thief ants. *Tetramorium caespitum* (L.), and *Myrmica* spp. (Latreille) were used as out-groups for the phylogenetic trees. The phylogenic tree (Fig. 2) showed that red imported fire ants, *S. invicta*, were more closely (68%) related to *S. molesta molesta* than to the other groups of thief ants from this study. An 82% homology for the sequences was calculated in MEGA 4 (p-distance = 0.18). The changes in the sequences were assumed to be made by (1) taxa joined together have descended from a common ancestor, (2) random mutation in nucleotides occurs in lineages over time, (3) the random mutation transpires at an approximately constant rate, and (4) the mutations are independent (Thorpe 1982). This is especially true when the species are minute or degrade with time in storage.

The use of COI sequences as DNA barcodes to identify unknown or undetermined species would greatly increase the efficiency of minute specimen of insects. For example, the revision of the thief ants by Pacheco (2007) listed 83 species. From a previous 149 available taxa, the author recognizes 72 valid species and identified 11 new species. COI sequence generated and the protocol used in this research could be reproduced on thief ant specimens collected in other locations. This could aid in identification of the species, reducing the difficulty associated with morphologic identification of such tiny ants.
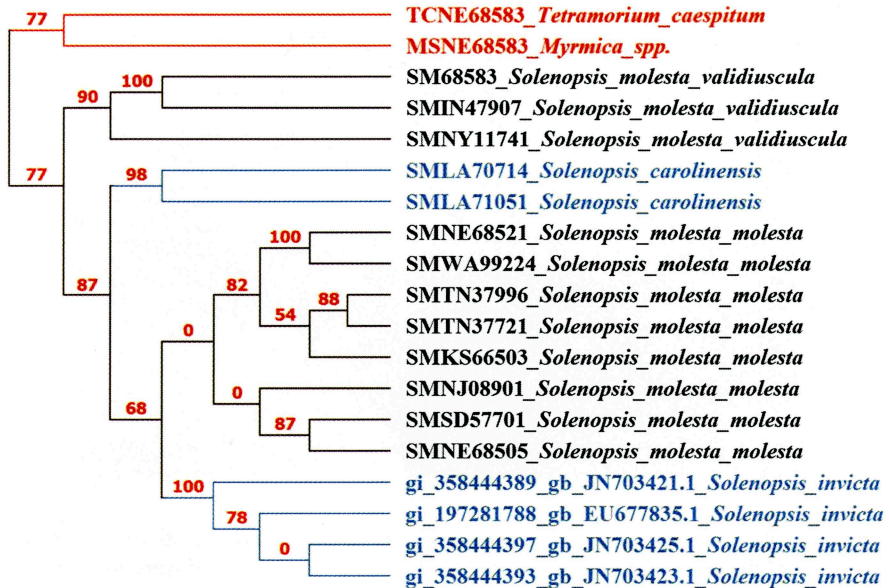
Fig. 2. Maximum Likelihood phylogenetic tree of thief ants collected from13 loca-
tions in 9 states across its distribution range; compared with red imported
fire ants; rooted with *Tetramorium caespitum* and *Myrmica* spp. Numbers
represent branch supporting values (as percent).

DNA barcode. Cytochrome oxidase subunit I (COI) sequences of thief ants from
13 locations in 9 states were submitted to GenBank. The frequency and percent of the
nucleotides were: A: 2859 (29.9%); C: 1824 (19.1%); G: 1253 (13.1%) and T: 3,612
(37.8%) (MEGA 4.0 Tamura et al. 2007). The sequences are comprised of 8,107 bp,
32.29% G+C content with an average length of 638 bp per submission. The COI
sequences of the thief ant are shown in Fig. 3. Periods in the sequence letters repre-
sents conserved bases between populations from each location. Conserved bases
are indicative of similarities between populations whereas the different bases account
for separation of the different populations within and between species.

These results indicate that a COI-based identification system could be effective in
identifying thief ants. These DNA barcodes could be used to determine related spe-
cies, to identify cryptic species (Hebert, et al. 2004, Burns, et al. 2008) or invasive
species (Rubinoff 2006, Darling and Blum 2007). The COI sequences DNA barcodes
generated during from this research is a valuable tool to be used in future research on
thief ants.

The GenBank accession numbers from HM179641 to HM179653 for the COI
sequences obtained from this study are presented in Table 2. The DNA sequences
associated with these accession numbers were used as DNA barcodes in this study.
The use of these DNA barcodes for identification would reduce or help rectify the dis-
crepancy associated with identification of this group of ants whereas enumerating the
number of species of ants in this group.

A

```
              10        20        30        40        50        60        70        80        90       100
          ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NE68583[HM179641]  ttatatttattttttgggacatgagcaggaacagttggaacaagcttaagaattttaattcgtacagaattaggaaaccccggatccttaattggaaatg
IN47907[HM179642]  ...................................c..................................................................
NY11741[HM179643]  ..t..aac..aaagatccagg.att.tt..tt..tcaat..g..taa.t.ttcg....gaa.tagg.tctcc....cc.ttaattaataatga.ca..t.t
LA70714[HM179644]  .....c....cc.a.cc.tc...t......t.a.c..ct..tcaa....t..aa.t....cctt...c....tcttgtaat..tc....caat..c..
LA71051[HM179645]  .....c....cc.a.cc.tc...t......t.a.c..ct..tcaa....t..aa.t....cctt...c....tcttgtaat..t....caat..c..
NE68521[HM179646]  ..........c.a.cc.tt...c....a.a.c..a.....tcaa....t..aa.t....ctt.........tcttgtaat..t....aat......
KS66503[HM179647]  ..........c.a.cc.tt...c....a.a.c..a.....tcaa....t..aa.t....cct.........tcttgtaat..t....aat......
NE68505[HM179648]  ..........c..cc.tt...c....a.t.a.c..a.....tcaa....t..aa.t....cct.........tcttgtaat.tc....aat......
SD57701[HM179649]  ..........c..cc.tt...c....a.t.a.c..a.....tcaa....t..aa.t....cct.........tcttgtaat..t....aat......
TN37721[HM179650]  ..........c.a.cc.tg...c....t.a.c..a.....tcaa....t..aa.t....cct.........tc.tgtaat..t....aat......
NJ08901[HM179651]  ..........t..c..cctt...c..a..t.a.c..a.....tattc.a....t..aa.t....cct.........tcttgtaat..t....aat......
TN37996[HM179652]  ..t.t..t.b.cc.cctttagctg.g.aa..at.ggt.tc..taaaa.ta.t....cccattg.att.ggatcctggggttctt.aa....a.atg.cca
WA99224[HM179653]  ..........t..c.acta.tgaag..c.a..t.a....t..tcaa....aa.t...ctt.........tcttgtaat..t....aat......
```

```
              110       120       130       140       150       160       170       180       190       200
          ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NE68583[HM179641]  atcaaatttatcacacgattgttacacgctcatgcatttattataattttttttatagttatacaattataattggaggatttggaaattgactagtacc
IN47907[HM179642]  ....................................................................................................
NY11741[HM179643]  t.a.ttcc.tagtt..t.g.ca.g..ct.at.at.a..t..t.t..ag..a.acctt....attgg.gg.t....aat..tt.gtgcctt..a..t
LA70714[HM179644]  ......c....tt.ct.a....taga....t.......................tt..............t..ttt....
LA71051[HM179645]  ......c....tt.ct.a....taga....t.......................tt..............t..ttt....
NE68521[HM179646]  ...........tt.cc.a....tagg....t..c.....................ct.c............t..ttt..c..
KS66503[HM179647]  ...........tt.cc.a....agg....c..c......................ct.............g........t..ttt..t..
NE68505[HM179648]  ...........tt.cc.a....aga....c..c......................ct.c............t..ttt..t..
SD57701[HM179649]  ....g......tt.cc.a....aga....c..a......................ct.............g........t..ttt..t..
TN37721[HM179650]  ...........tt.cc.a....agg....c..c......................ct.............g........t..ttt..t..
NJ08901[HM179651]  ...........tt.cc.a....aga....c..a......................ct.............g........t..ttt..t..
TN37996[HM179652]  .attt..aat.ccata.t.acaacggcatg.c.tt..ataat.tt....a.agt..tgcccttt.taat.gggg..tttggaaatttt..agt.cccttt
WA99224[HM179653]  ...........tt.cc.a....taga....tg.c....................ct.c..............t..ttt...c..
```

```
              210       220       230       240       250       260       270       280       290       300
          ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NE68583[HM179641]  actaatactaggtgccctgatatagctttctcotcgtgtttaaataataataagattttgacttccttccccccttcctttgtcacttcttttaataagaagaata
IN47907[HM179642]  ..........t...a.t...................................................................................
NY11741[HM179643]  cgg.tct.c..a.atag.ct.ccc.cg.a.aaa.aa.a...gatt.tg.cttc.cccc.c.tc..taa.cct.c.tattt.aagaag.t.tt.actcg
LA70714[HM179644]  c........c..at.t..c....ac....ca....c.........c........c........a..c.ca.t..t.aa.tt..g....t.t
LA71051[HM179645]  c........c..at.t..c....ac....ca....c.........c........c........a..c.ca.t..t.aa.tt..g....t.t
NE68521[HM179646]  ..t.......c..gt.t.........c.a....a................................t.a......a..t.aa.tt..g....t.t
KS66503[HM179647]  c..t.......c......c.ac....a.............................t.g....a....a.t..t.aa.tt..g....t.t
NE68505[HM179648]  t..t......t..at.........c.a....a................................t.g....a..cc.aa.t..t.aa.tt..g....t.t
SD57701[HM179649]  tt.t......t..at.........c.a....a................................t.g....a..c.aa.t..t.aa.tt..g....t.t
TN37721[HM179650]  c..t.......c......c.ac....a................................t.a....a..c.aa.t..t.aa.tt..g....t.t
NJ08901[HM179651]  t..t......c..gt.........c.a....a................................t.a......a..cc.aa.t..t.aa.tt..g....t.t
TN37996[HM179652]  .aattaggggcccccagatat.gc.ta.cc.cgtataaaa..tat.agatttttgac.tttac.cc..t.t.....t.tttaat...a.ga.gattt.t..
WA99224[HM179653]  ...c.......c..gt.t.........c.a....a................................t.a......a..c.aa.t..t.aa.tt..g....t.t
```
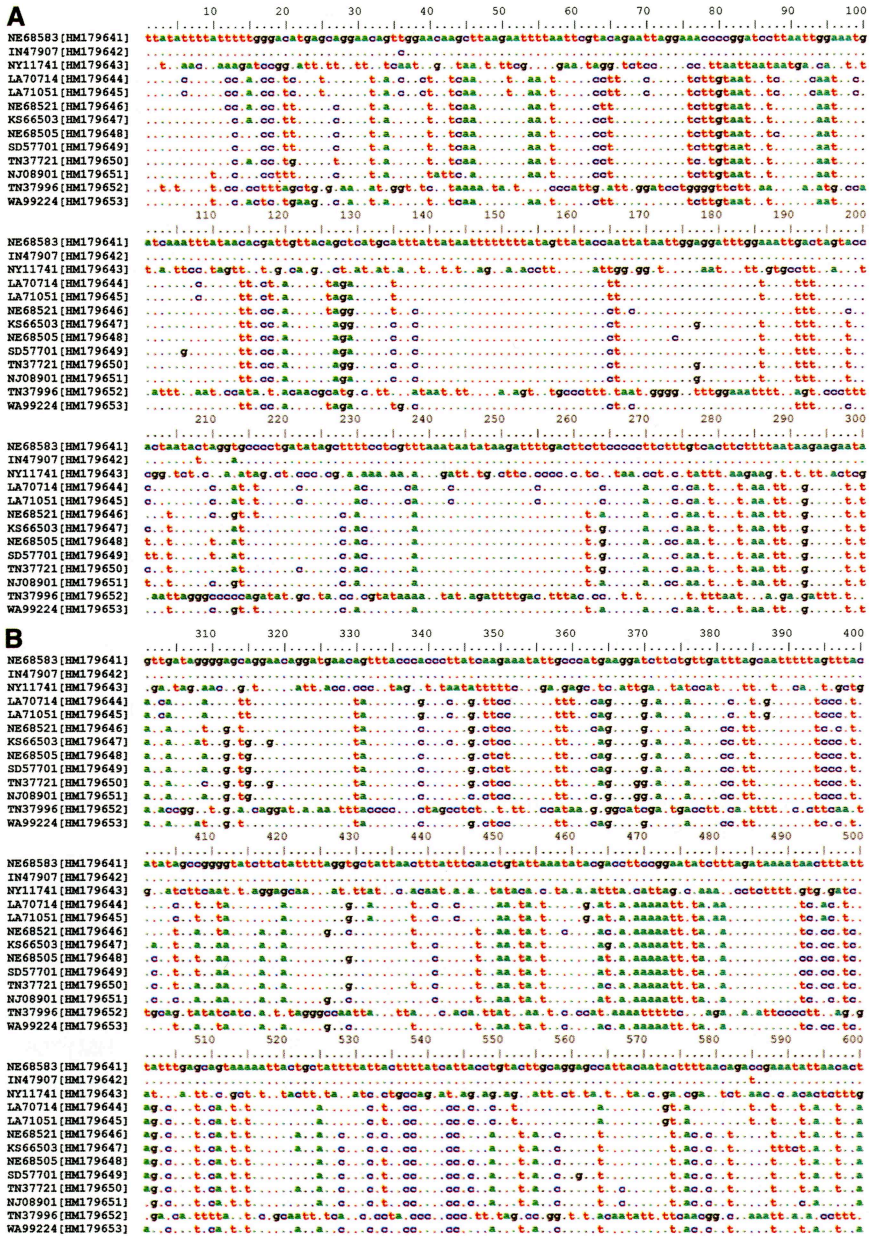
B

```
              310       320       330       340       350       360       370       380       390       400
          ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NE68583[HM179641]  gttgataggggagcaggaacaggatgaacagtttaccccacccttatcaagaaatattggccatgaaggatcttctgttgatttagcaattttttagttttac
IN47907[HM179642]  ....................................................................................................
NY11741[HM179643]  .ga.tag.aac..g.t.....att.acc.ccc..tag..t.taatatttttc...ga.gagc.tc.attga..tatccat....tt..t..ca..t.gctg
LA70714[HM179644]  a.ca.....a...tt...........ta......g...c..g.ttcc....ttt..cag...g.a...a....c..t.g.....tccc.t.
LA71051[HM179645]  a.ca.....a...tt...........ta......g...c..g.ttcc....ttt..cag...g.a...a....c..t.g.....tccc.t.
NE68521[HM179646]  a..a...t..g.t...........ta......c.........g.ctcc....tt..cag...g.....a....cc.tt.....tc.c.t.
KS66503[HM179647]  a..a....at..g.tg..g...........ta......c..g.g.ctcc....tt....ag...g.a...a....cc.tt.....tccc.t.
NE68505[HM179648]  a..a...a..g.tg...........ta......c..g.g.ctcc....tt..cag...g.a...a....cc.tt.....tccc.t.
SD57701[HM179649]  ..t..a....a..g.tg..g...........ta......c..g.g.ctct....tt..cag...g.a...a....cc.tt.....tccc.t.
TN37721[HM179650]  a..a....c..g.tg..g...........ta......c..g.g.ctcc....tt....ag...gg.a...a....cc.tt.....tccc.t.
NJ08901[HM179651]  a..a...a..g.tg...........ta......c..g.c.ctcc....tt..c.g..gg.a...a....cc.tt.....tccc.t.
TN37996[HM179652]  a.accgg..t.g.a.caggat.a.aa.tttacccc...ctagcctct..t.tt..ccataa.g.ggcatcga.tgacctt.ca.tttt..c.cttcaa.t
WA99224[HM179653]  a..a...at..g.t...........ta......g.ctcc....tt..cag...g.....a....cc.tt.....tc.c.t.
```

```
              410       420       430       440       450       460       470       480       490       500
          ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NE68583[HM179641]  atatagccgggggtatcttctatttttaggtgctgctattaacttttattcaactgtattaaatatacgacccttcacggaatatcttttagatataaataacttttatt
IN47907[HM179642]  ....................................................................................................
NY11741[HM179643]  g..atcttcaat.t.aggagcaa...at.ttat..c.acaat.a.a..tatcca.c.ta.a.atttta.cattag.c.aaa..cctctttt.gtg.gatc.
LA70714[HM179644]  ...c.t..ta.......a...........t..c..c....aa.ta.t....g.at.a.aaaaatt.ta.aa.......tc.ac.t...
LA71051[HM179645]  ...c.t..ta.......a...........t..c..c....aa.ta.t....g.at.a.aaaaatt.ta.aa.......tc.ac.t...
NE68521[HM179646]  ...t.b.ta.....a..a.....g.c........t..c..aa.ta.t..c....ac.a.aaaaatt.ta..a.......tc.cc.tc.
KS66503[HM179647]  .a..t..a..aa.....a...........t.c.c....t.b.aa.ta.t....a.ag.a.aaaaatt.ta..a.......tc.cc.tc.
NE68505[HM179648]  .c..t..aa.....a...........g........t..c..aa.ta.t....at.a.aaaaatt.ta..a.......cc.cc.tc.
SD57701[HM179649]  .c..t..aa.....a...........c........c.....aa.ta.t....ac.a.aaaaatt.ta..a.......cc.cc.tc.
TN37721[HM179650]  .c..t..aa.....a..a.....g.c........t..c..aa.ta.t....ac.a.aaaaatt.ta..a.......tc.cc.tc.
NJ08901[HM179651]  .c..c..a..aa.....a.....g.c........c........aa.ta.t....at.a.aaaaatt.ta..a.......tc..c.tc.
TN37996[HM179652]  tgcag.tatatcatc.a.t.tagggcaaatta..tta...c.aca.ttat..aa.t.c.ccat.aaaattttc...aga...a.attcccctt..ag.g
WA99224[HM179653]  .c..t..a..ta.....a..a.....g.c........t..c..aa.ta.t....ac.a.aaaaatt.ta..a.......tc.cc.tc.
```

```
              510       520       530       540       550       560       570       580       590       600
          ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NE68583[HM179641]  tatttgagcagtaaaaattactgctatttttattacttttatcattacctgtacttgcaggagccattacaatactttttaacagacacgaaatattaacact
IN47907[HM179642]  ............................................................................................t.......
NY11741[HM179643]  at...a..tt.c.gct.t..tacatt.ta..atc.ctgccag.at.ag.ag.ag..att.ct.ta.t..ta.c.ga.cga..tct.aac.c.acactctttg
LA70714[HM179644]  ag.c....t.ca.t.t.........a......c...cc.c..c..t...............a.......gt.a.......t..t.t..a..t..a
LA71051[HM179645]  ag.c....t.ca.t.t.........a......c.t.cc....cc.c..c..t...............a.......gt.a.......t..t.t..a..t..a
NE68521[HM179646]  ag.c....t.ca.t.t.........a..a...c...cc.cc.cc...........t.a..c.......t.ac.c..t........t..t.a..t..a
KS66503[HM179647]  ag.c....t.ca.t.t.........a..a...c...cc.c..cc...........t.a..c...........t.ac.c..t......tttct..a..t..a
NE68505[HM179648]  ag.c....t.ca.t.t.........a......c...c.t.cc...cc.a..........t.a..c.......t.ac.c..t........t..t.a..t..a
SD57701[HM179649]  ag.c....t.ca.t.t.........a......c...cc...cc.a..t.a..c..g.t........t.ac.c..t........t..t.t..a
TN37721[HM179650]  ag.c....t.ca.t.t.........a..a...c...cc...cc.a..........t.a......c..t........t.ac.c..t........t..t.t..a
NJ08901[HM179651]  .g.c....t.ca.t.t.........a......c...c.t.cc...cc...........t.c.......t.ac.c..t........t..t.t..a
TN37996[HM179652]  .ga.ca.tttta..t.c.gcaatt.tca...c.ccta.ccc....ccc.tt.tag.cc.gg.t.t.acaatatt.ttcaacgg.c..aaatt.a.a.ccttt.
WA99224[HM179653]  .a.c....t.ca.t.t.........a..a...c...cc....cc...........a..t.a..c...........t.ac.c..t........t..t.a..t..a
```

Fig. 3. Thief ants: States, Accession numbers and base sequences with an average length of 638 bp. Periods between bases represented conserved bases. (Ant collection locations: IN = Indiana, KS = Kansas, LA = Louisiana, NE = Nebraska, NJ = New Jersey, NY = New York, SD = South Dakota, TN = Tennessee, WA = Washington followed by zip codes).

C

```
                            610       620       630
                   ....|....|....|....|....|....|....|...
NE68583[HM179641]  tcatttttgaccctgccggaggaggagacc
IN47907[HM179642]  ..c..............t..g..........caatttt
NY11741[HM179643]  atcc.agag..ggag.agaccca.tt.t
LA70714[HM179644]  .....c.....t...t.a...........t.ctattct
LA71051[HM179645]  .....c.....t...t.a...........t.ctattct
NE68521[HM179646]  ..t..c..c..t...t.......g..g....caatttt
KS66503[HM179647]  ..t..c.....t..ct.g.....g.......caatttt
NE68505[HM179648]  ..t..c..c..t..ct.......g.......caatttt
SD57701[HM179649]  ..t..c..c.....ct.a.....g.......caatttt
TN37721[HM179650]  ..t..c..c..t..ct.g.....g...a..caacata
NJ08901[HM179651]  ..t..c.....t..ct.a.....g.......caattt
TN37996[HM179652]  .tt..cccccc.t.ccgg.ag.cgacca.aatga
WA99224[HM179653]  ..t..c..c..t...t.....a.g..tt...caataaa
```

Fig. 3. Continued

Conclusions

The DNA barcodes generated from COI sequences of thief ant species/subspecies and deposited in GenBank could be used by other researchers to differentiate these species. The same methodology and protocols could be used or modified to generate DNA barcodes for other ant species that are difficult to identify via the dichotomous keys. Specimens that are very minute, such as thief ants, or disintegrated due to age could be identified once COI sequences from previously identified specimens have been sequenced and the sequences deposited in gene banks.

Table 2. GenBank accession numbers for identified thief ant specimens from 13 collection sites.

State	Zip code	Specimen Identification	Accession #
Nebraska	68583	*Solenopsis molesta validiuscula*	HM179641
Indiana	47907	*Solenopsis molesta validiuscula*	HM179642
New York	11741	*Solenopsis molesta validiuscula*	HM179643
Louisiana	70714	*Solenopsis carolinensis*	HM179644
Louisiana	71051	*Solenopsis. carolinensis*	HM179645
Nebraska	68521	*Solenopsis molesta molesta*	HM179646
Kansas	66503	*Solenopsis molesta molesta*	HM179647
Nebraska	68505	*Solenopsis molesta molesta*	HM179648
South Dakota	57701	*Solenopsis molesta molesta*	HM179649
Tennessee	37721	*Solenopsis molesta molesta*	HM179650
New Jersey	08901	*Solenopsis molesta molesta*	HM179651
Tennessee	37996	*Solenopsis molesta molesta*	HM179652
Washington	99224	*Solenopsis molesta molesta*	HM179653

Based on literature reviewed, this is the first submission of COI generated DNA barcodes for thief ants to GenBank. This would help to identify thief ants in other states and also determine the number of thief ant species (subspecies) found within the USA and possibly identify new species of thief ants. The COI sequences DNA barcodes generated would facilitate easier identification of each species and reduce the conflict generated when morphological identification is used to separate specimen.

Acknowledgments

References Cited

Bennett, G. W., J. M. Owens and R. M. Corrigan. 2005. Truman's scientific guide to pest management operations. 6th ed. Purdue Univ./Questex Media. West Lafayette, IN.

Bickford, D., D. J. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. K. Ingram and I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22: 148-155.

Burns, J. M., D. H. Janzen, M. Hajibabaei, W. Hallwachs and P. D. N. Hebert. 2008. DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. Proc. Natl. Acad. Sci. USA 105: 6350-6355.

Chown, S. L., B. J. Sinclair and B. J. van Vuuren. 2008. DNA barcoding and the documentation of alien species establishment on sub-Antarctic Marion Island. Polar Biol. 31: 651-655.

Chu, K. H., M. Xu and C. P. Li. 2009. Rapid DNA barcoding analysis of large datasets using the composition vector method. BMC Bioinformatics 10(Suppl. 14): S8.

Darling, J. A. and M. J. Blum. 2007. DNA-based methods for monitoring invasive species: a review and prospectus. J. Biolog. Invasions 9: 751-765.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.M. Claverie and O. Gascuel. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008 Jul 1;36 (Web Server issue): W465-9. Epub. 2008, April19.

Field, H. C., W. E. Evans Sr., R. Hartley, L. D. Hansen and J. H. Klotz. 2007. A survey of structural ant pests in the southwestern U.S.A. (Hymenoptera: Formicidae). Sociobiol. 49: 151-164.

Fournier, D., D. DuBios and S. Aron. 2008. Isolation and characterization of microsatellite loci from the invasive ant *Pheidole megacephala*. Mol. Ecol. Resour. 8: 919-922.

Hajibabaei, M., D. H. Janzen, J. M. Burns, W. Hallwachs and P. D. N. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. USA 103: 968-971.

Hajibabaei, M., G. A. C. Singer, P. D. N. Hebert and D. A. Hickey. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends Genet. 23: 167-172.

Hebert, P. D. N., A. Cywinska, S. L. Ball and J. R. deWaard. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B Biol. Sci. 270: 313-321.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA 101: 14812-14817.

Hurst, G. D. D. and F. M. Jiggins. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. Biol. Sci. 272: 1525-1534.

Husen, T. J., N. A. Spomer, R. Narain, S. T. Kamble, D. Branscome, E. Roper and B. Cartwright. 2008. Efficacy of Optigard® Flex, Termidor® SC, and I MaxxPro® 2F against selected nuisance ants (odorous house, pavement, and thief) when applied as perimeter treatments. Arthrop. Manag. Tests 34: 6.

Hye, S. Y., E. Jae-Yong, S. K. Jong, K. Young-Jun, M. Mi-Sook, K. P. Woon, L. Hang and K. Chang-Bae. 2006. DNA barcoding Korean birds. Mol. Cells 22: 323-327.

Kerr, K. C. R., M. Y. Stoeckle, C. J. Dove, L. A. Weigt, C. M. Francis and P. D. N. Hebert. 2007. Comprehensive DNA barcode coverage of North American birds. Mol. Ecol. Notes 7: 535-543.

Klotz, J., L. Hansen, R. Pospischil and M. Rust. 2008. Urban ants of North America and Europe: identification, biology and management. Cornell Univ. Press. Ithaca, NY.

Lahaye, R., M. van der Bank, D. Bogarin, J. Warner, F. Pupulin, G. Gigot, O. Maurin, S. Duthoit, T.G. Barraclough, and V. Savolainen. 2008. DNA barcoding the floras of biodiversity hotspots. Proc. Natl. Acad. Sci. USA 105: 2923-2928.

Narain, R. B., S. T. Kamble and T. O. Powers. 2012. Morphological characterization and molecular mediated genetic variation of thief ants (Hymenoptera: Formicidae). Sociobiol. 59: 617-631.

Pacheco, J. A. 2007. The New World thief ants of the genus *Solenopsis* (Hymenoptera: Formicidae). Ph. D. Dissert. University of Texas-El Paso, El Paso.

Rohfritsch, A. and P. Borsa. 2005. Genetic structure of Indian scad mackerel *Decapterus russelli*: Pleistocene vicariance and secondary contact in the Central Indo-West Pacific Seas. Heredity 95: 315-326.

Rubinoff, D. 2006. Utility of mitochondrial DNA barcodes in species. Conserv. Biol. 20: 1026-1033.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Smith, M. A., D. M. Wood, D. H. Janzen, W. Hallwachs and P. D. N. Herbert. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. Proc. Natl. Acad. Sci. USA 104: 4967-4972.

Smith, M. A., N. E. Woodley, D. H. Janzen, W. Hallwachs and P. D. N. Hebert. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proc. Natl. Acad. Sci. USA 103: 3657-3662.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Tavares, E. S. and A. J. Baker. 2008. Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. BMC Evol. Biol. 8: 81.

Thorpe, J. P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annu. Rev. Ecol. Syst. 13: 139-168.

Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last and P. D. N. Hebert. 2005. DNA barcoding Australia's fish species. Philosoph. Trans. R. Soc. B-Biol. Sci. 360: 1847-1857.

Whitworth, T. L., R. D. Dawson, H. Magalon and E. Baudry. 2007. DNA barcoding cannot reliably identify species of the blowfly genus *Protocalliphora* (Diptera: Calliphoridae). Proc. Biol. Sci. 274: 1731-1739.