**The Great Lakes Entomologist**

Volume 42
Numbers 1 & 2 - Spring/Summer 2009 *Numbers 1 & 2 - Spring/Summer 2009*
Article 2

April 2009

# The Alien *Hippodamia Variegata* (Coleoptera: Coccinellidae) Quickly Establishes Itself Throughout Wisconsin

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*Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is a Palaearctic species that was first reported to be established in the Nearctic near Montreal, Quebec, in 1984 (Gordon 1987). Since then, this small beetle has been expanding its range in North America, a process summarized by Gardiner and Parsons (2005). It was first reported from Michigan in 2005 (Gardiner and Parsons 2005) and from Ohio in 2007 (Pavuk *et al*. 2007).

Daniel Young was the first to collect this beetle in Wisconsin, finding it on 29 July 2005 in Sheboygan County. Andrew Williams found this species on 2 August 2005, and has been casually collecting specimens to voucher its presence in Wisconsin counties. In 2005, Williams collected it in Dane, Jefferson, Portage, and Waukesha Counties. In 2006, he collected it in Kenosha and Rock Counties. All of these counties are in the southeastern quarter of Wisconsin. He did not collect it in 2007. In 2008, Williams collected it in Brown, Calumet, Door, Dunn and Juneau Counties, which lie farther north and/or farther west. In 2009, he collected it in Ashland, Buffalo, Florence, Iron, Sawyer, St. Croix, and Washburn Counties, which include peripheral counties as far north and as far west as occur in Wisconsin. All of this collecting was serendipitous. It shows that in just five years, or perhaps even more quickly, this alien coccinellid has swept across all of Wisconsin.

*Hippodamia variegata* is not the first alien coccinellid to do so. *Coccinella septempunctata* (Linnaeus) and *Harmonia axyridis* (Pallas), two rather large coccinellids, can now be easily found throughout Wisconsin. Indeed, these three alien coccinellid species are the lady beetles we most frequently find, by far, while searching herbaceous vegetation in the course of other research projects, as well as in general collecting. What impacts are these alien lady beetles having on our native coccinellids, aphids of non-economic importance, and other fauna? Koch (2003) provided a cogent review of initial research in this area. Our chief concern is the threat to native biodiversity, which will likely never be adequately researched. We think the continued policy of deliberate importation and release of such generalist predators to address the challenges posed by particular agronomic pests is unwise.

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# Description of a New Species of *Atanycolus* (Hymenoptera: Braconidae) from Michigan Reared from the Emerald Ash Borer, *Agrilus planipennis* (Coleoptera: Buprestidae: Agrilinae)

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**Abstract**

A new species of Braconidae, *Atanycolus cappaerti* Marsh and Strazanac, is described from Michigan where it has been reared from emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), galleries in ash (*Fraxinus* spp.). Molecular evidence (analysis of fragments of the mitochondrial genes COI and 16S and the nuclear genes *wingless* and elongation factor 1-alpha) is presented to support the distinction of this new species from *A. hicoriae* Shenefelt that has been documented as a parasitoid of several ash borers.

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In 2002 a new exotic forest pest, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), the emerald ash borer (EAB), was discovered for the first time in Michigan and Ontario (Haack et al. 2002, Herms et al. 2004, EAB Info 2009). This pest is native to northeastern China, Korea, Mongolia, Japan, Taiwan, and the Russian Far East. In China, the only recorded hosts are several species of ash (*Fraxinus*) but it has been recorded from species of *Pterocarya* and *Ulmus* in other parts of its native range. In North America, EAB has spread from Michigan and Ontario to Indiana, Illinois, Missouri, Ohio, Wisconsin, Minnesota, Pennsylvania, West Virginia, Kentucky, New York, Virginia, Maryland (nursery stock), and Quebec and has been found to attack all native species of ash (EAB Info 2009).

In 2005, specimens of the braconid genus *Atanycolus* Foerster reared from green ash (*Fraxinus pennsylvanica* Marsh.) infested with EAB in Plymouth, Michigan were sent to PMM, who identified them as *A. hicoriae* Shenefelt. In 2007 and 2008 specimens were again sent to PMM which were reared from EAB galleries in ash at Fenton, Michigan; they were identified as *A.* sp. 1 nr. *hicoriae* and *A.* sp. 2 nr. *hicoriae* (specimen labeled as *A.?hicoriae*). Additionally, there was some ecological evidence supporting differences between *A. hicoriae* (Plymouth, MI) and *A.* sp. nr. *hicoriae* (Fenton, MI) (D. Cappaert, pers. comm.). Subsequent molecular analysis was carried out by SYL to find evidence to support the possibility that the Fenton specimens represented a new species different from those from Plymouth identified as *A. hicoriae*.

The purpose of this paper is to provide a name for a new species of *Atanycolus* for workers studying its biology and potential as a biocontrol agent against EAB in Michigan.

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## Materials and Methods

For the descriptive part, specimens were examined using a Wild M5 stereomicroscope. Available type material for all North American species of *Atanycolus* were studied and compared with the new species. Scanning electron micrographs were made using the Hitachi S-3500N scanning electron microscope at the Department of Entomology, Kansas State University. The SEM images and wing photograph were enhanced and plate composed using Adobe Photoshop (by JSS). Most of the morphological terminology used follows that proposed by Harris (1979) and Marsh (2002).

For the molecular analysis, four genes were included in this study for their phylogenetic utility in previous braconid research: mitochondrial 16S rDNA (16S) (Michel-Salzat and Whitfield 2004; Whitfield et al. 2002), mitochondrial cytochrome *c* oxidase subunit I (COI) (Whitfield et al. 2002), nuclear protein coding *wingless* (*wg*) (Banks and Whitfield 2006), and nuclear protein coding elongation factor 1-alpha F2 copy (EF1a) (Banks and Whitfield 2006).

The insects used in this study were collected in the field. *Digonogastra* sp., *Atanycolus simplex*, *A.* sp.1 nr. *simplex* (labeled as *A.*?*simplex*), and *A. ulmicola* 2 (Illinois) were identified by Jim Whitfield. *Atanycolus hicoriae*, *A.* sp. 1 nr. *hicoriae*, *A.* sp. 2 nr. *hicoriae*, *A. ulmicola* 1 (West Virginia), and *Doryctes* sp. were identified by Paul Marsh. The origin of the nine specimens included in this study is provided in Table 1. *Atanycolus* sp. 1 nr. *hicoriae*, *A.* sp. 2 nr. *hicoriae*, *A. ulmicola* 1 (West Virginia), *Digonogastra* sp., and *Doryctes* sp. were preserved in ethanol until DNA was extracted. *Atanycolus hicoriae*, *A. simplex*, *A.* sp. 1. nr. *simplex*, and *A. ulmicola* 2 (Illinois) were dried, pinned specimens. A DNeasy tissue extraction kit (QIAGEN Inc., Valencia, CA, USA) was used to extract the DNA. Voucher material of specimen remnants are located in the Whitfield Systematics Laboratory at the University of Illinois, Urbana, and DNA sequences obtained were deposited in GenBank. Polymerase chain reactions (PCR) were executed with an Eppendorf Mastercycler thermocycler (Eppendorf AG, Westbury, NY, USA). PCR consisted of 5µl of 10x Hotmaster *Taq* buffer (5 Prime Inc., Gaithersburg, MD, USA), 5 µl of dNTPs (8 mM), 5 µl of the forward and reverse primers (2.5-10 mM), 0.125 µl of Hotmaster *Taq*, 2.5-5 µl of DNA, and 24.75-27.25 µl of molecular biology grade water. The total reaction volume was 50 µl. The primers used are provided in Table 2. The lengths and temperatures of each PCR step are located in Table 3.

Table 1. Label information of specimens used in the study.

| Specimen | Label Information |
|---|---|
| *A. hicoriae* | Michigan: Plymouth 04/19/2005 FH139(4-5) |
| *A.* sp. nr. *hicoriae* | Michigan: Fenton 7 Lakes State Park March/April 2008 |
| *A.* ?*hicoriae* | Michigan: Fenton 7 Lakes State Park March 2008 |
| *A. ulmicola* 1 | West Virginia: Hardy Co. V-17 to VI-6-05 |
| *Digonogastra* sp. | Rio Blanco Abajo, Sector San Cristobal, Area de Conservacion Guanacaste, Alajuela Province, Costa Rica lat 10.90037 long -85.37254 elev 500m 18-24-I-2008 |
| *Doryctes* sp. | West Virginia: Hardy Co. VIII-12-27-2003 |
| *A. simplex* | Illinois: Champaign Co. Busey Woods (BioBlitz) 24-25-VI-2005 |
| *A.* ?*simplex* | Arkansas: Wash. Co. Mt. Sequoyah 600m 1-16-VII-1993 |
| *A. ulmicola* 2 | Illinois: Champaign Co. Busey Woods (BioBlitz) 24-25-VI-2005 |

Table 2. Primers used in this study.

| Gene | Primer | Sequence | Annealing Temperature | Reference |
|---|---|---|---|---|
| 16S | | | 52-57°C | |
| Forward | 16S outer | CTTATTCAACATCGAGGTC | | (Whitfield 1997) |
| Reverse | 16SWb | CACCTGTTTATCAAAACAT | | (Dowton and Austin 1994) |
| COI | | | 50-53°C | |
| Forward | LCO1490 | GGTCAACAAATCATAAAGATATTGG | | (Folmer et al. 1994) |
| Reverse | HCO2198 | TAAACTTCAGGGTGACCAAAAAATCA | | (Folmer et al. 1994) |
| EF1a | | | 47-52°C | |
| Forward | EF1A1F | AGATGGGYAARGGTTCCTTCAA | | (Belshaw and Quicke 1997) |
| Reverse | EF1A1R | AACATGTTGTCDCCGTGCCATCC | | (Belshaw and Quicke 1997) |
| *Wg* | | | 47-53°C | |
| Forward | Wg1 | GARTGYAARTGYCAYGGYATGTCTGG | | (Brower and DeSalle 1998) |
| Reverse | Wg2 | ACTICGCRCACCARTGGAATGTRCA | | (Brower and DeSalle 1998) |

Table 3. Temperatures and lengths of denaturation, annealing and elongation steps in PCR.

| Gene | Initial Denaturation | Denaturation | Annealing | Elongation | Final Elongation | Cycles |
|---|---|---|---|---|---|---|
| COI | 94°C | 94°C | 50°C | 68°C | 72°C | 35 |
| | 5:00 | 1:00 | 1:00 | 1:00 | 5:00 | |
| 16S | 94-95°C | 94-95°C | 47-48°C | 68°C | 68-72°C | 31-35 |
| | 1:00-3:00 | 0:45-1:00 | 1:00 | 1:00-3:00 | 4:00-5:00 | |
| *Wg* | 94-95°C | 94-95°C | 48-52°C | 65°C | 65°C | 34 |
| | 2:00 | 0:20 | 0:20 | 1:00 | 5:00 | |
| EF1a | 94°C | 94°C | 48-51°C | 65°C | 65°C | 34-35 |
| | 2:00 | 0:20 | 0:20 | 1:00 | 5:00 | |

PCR products were purified using a Qiaquick purification kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's protocol. PCR products were sequenced at the University of Illinois W. M. Keck Center. Sequences were edited using Sequencher version 4.7 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned in BioEdit version 7.0.x (Hall 1999) using the Clustal W algorithm (Thompson et al. 1994).

Phylogenetic reconstruction for all of the data used Mr. Bayes 3.1.2 (Huelsenbeck and Ronquist 2001) and ran for 10 million generations. Dirichlet priors were used initially for the state frequencies and each was modeled separately based on a GTR + inv + gamma model (nst=6, rates=invgamma). Sequence divergences for COI and 16S were calculated with the Kimura 2 Parameter distance model (Kimura 1980) in PAUP* 4.0b10 (Swofford 2003). PAUP* was also used to generate neighbor joining trees to graphically represent the distance between sequences. Neighbor joining trees generated in PAUP* were imported into and edited in Dendroscope (Huson et al. 2007).

## Results and Discussion

*Atanycolus* sp. 1 nr. *hicoriae* and *A.* sp. 2 nr. *hicoriae* have virtually identical COI sequences (0.3% sequence divergence = 2 base pairs out of 615) and identical 16S sequences (no sequence divergence), indicating they are most likely the same species. *Atanycolus* sp. 1 nr. *hicoriae*, *A.* sp. 2 nr. *hicoriae* and *A. hicoriae* have 7% COI sequence divergence (42 base pairs out of 615) suggestive of a different species. Brower (1994) compiled and plotted divergence data from a variety of insects that showed a typical rate of divergence of 1.5-2.5% per million years in mtDNA (including COI). Thus, assuming a similar rate of substitution in *Atanycolus*, a divergence of 7% would correspond to a divergence of roughly 3 million years for these essentially sympatric individuals. Even accepting the fact that evolutionary rates vary somewhat from group to group, it is difficult to conceive how they could be conspecific with a divergence level of 7%.

The sequence divergence between COI sequences was additionally supported by divergences in 16S (2% sequence divergence = 10 base pairs out of 413); the two nuclear genes found little divergence among any of the species (even previously accepted ones) as they are more strongly conserved. The Bayesian mixed-model analysis found the two putative *A. cappaerti* grouped with 1.0 posterior probability, and these two grouped with *A. hicoriae* also at 1.0 posterior probability. The same level of posterior probability linked the three *A. ulmicola* specimens into a single clade. *Atanycolus simplex* appeared as sister to the *A. hicoriae* + *A. cappaerti* clade, but only with 0.67 posterior probability. Graphical representation of the total Kimura 2 parameter distances between combined sequences for the 4 genes is presented as a neighbor joining tree in Figure 1.

Sequences have been deposited in GenBank under accession numbers GU135642-GU135650 (16S), GU173825-GU173833 (COI), GU173815-GU173820 (EF1a) and GU173821-GU173824 (*wg*).

### Genus Atanycolus Foerster

The genus *Atanycolus* is easily distinguished from other members of the subfamily Braconinae by the modified antennal scape with the excavations at its base and apex. It can be identified by the keys provided by Quicke and Sharkey (1989) and Quicke (1997). A review of the North American species is in preparation (Marsh and Strazanac, in prep.) in which a revised key, description and synonymies will be presented for all North American species.

### *Atanycolus cappaerti* Marsh and Strazanac, new species

**Diagnosis**. *Atanycolus cappaerti* is very similar morphologically to *A. hicoriae* and will run with difficulty to that species in the key presented by

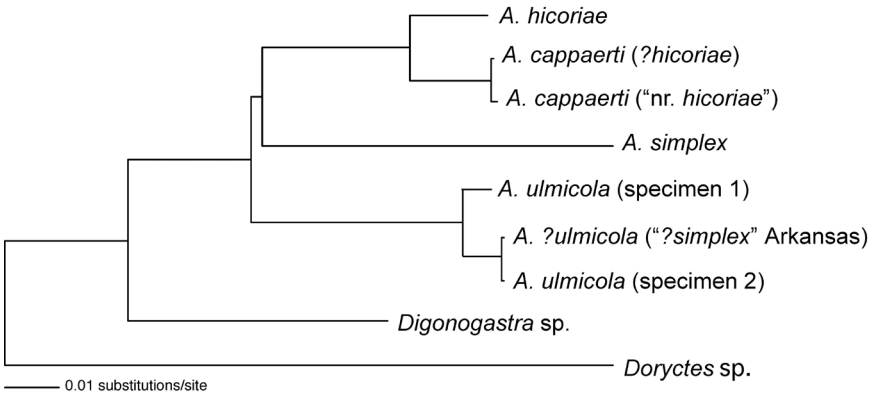


Figure 1. Neighbor joining tree (K2P distances) for all specimens and the 4 genes.

Shenefelt (1943). The differences between the two species are subtle and are as follows. The metasomal terga of *A. cappaerti* are usually red with infusions of pink and the venter of the metasoma is always pinkish red; the metasoma of *hicoriae* is yellow or yellowish red. The apical flagellomeres (behind the apical most one) of *A. cappaerti* are slightly longer than wide (Fig, 2F); the apical flagellomeres of *A. hicoriae* are as wide as or wider than long (Fig. 2G). The apical flagellomere of *A. cappaerti* is usually sharply pointed and 1.5 times as long as the penultimate flagellomere, whereas the apical flagellomere of *A. hicoriae* is usually roundly pointed and less than 1.5 times as long as penultimate flagellomere, but this character is variable and not easily seen. The antenna of *A. cappaerti* usually has less than 40 flagellomeres whereas that of *A. hicoriae* usually has more than 40 flagellomeres. The wings of *A. cappaerti* are smokey grey whereas those of *A. hicoriae* are usually dusky brown.

**Female.** *Color*: head black, mandibles orange medially, rarely eye orbits partially orange; antenna black, rarely weakly orange on inner side at apex of pedicel and scape; mesosoma black, rarely propodeum with brown at apex; fore and hind wings smokey grey, fore wing with transverse hyaline line across middle of first submarginal cell and hyaline spot in second discal cell below junction of veins m-cu, 3RS and 3M (Fig. 2D); legs black, second trochanters usually orange at apex; metasomal terga reddish, usually with pink hue, median plate of first tergum often orange, ventrally metasoma always pinkish red; ovipositor sheaths black, ovipositor light brown. *Size*: body length, 5-7 mm; fore wing length, 4-6 mm; ovipositor length, 4-6 mm. *Head*: broad but flattened dorsoventrally, width across eyes greater than height; face (Fig. 2A) smooth, often slightly excavated above clypeus and with distinct swelling below antennal bases, numerous setal punctures laterally; clypeus concave medially; malar space 1/5-1/6 eye height; frons (Fig. 2B) excavated, with groove around ocellar triangle and extending to between antennae; vertex (Fig. 2B) smooth, ocell-ocular distance slightly less than diameter of lateral ocellus; temple smooth, width equal to eye width; antenna usually with 35-38 flagellomeres, rarely with 40; scape (Fig. 2C) about twice as long as wide, with distinct deep excavation anteriorly at apex; apical flagellomere (Fig. 2F) sharply pointed and about 1.5 times longer than penultimate flagellomere, all flagellomeres longer than wide, apical ones slightly so. *Mesosoma* (Fig. 2H): smooth and shining, somewhat flattened dorsoventrally; notauli indicated by row of setae and rarely barely impressed anteriorly; scutellum slightly raised, with scattered setae across apical border, prescutellar furrow distinctly scrobiculate; mesopleuron often with shallow curved groove

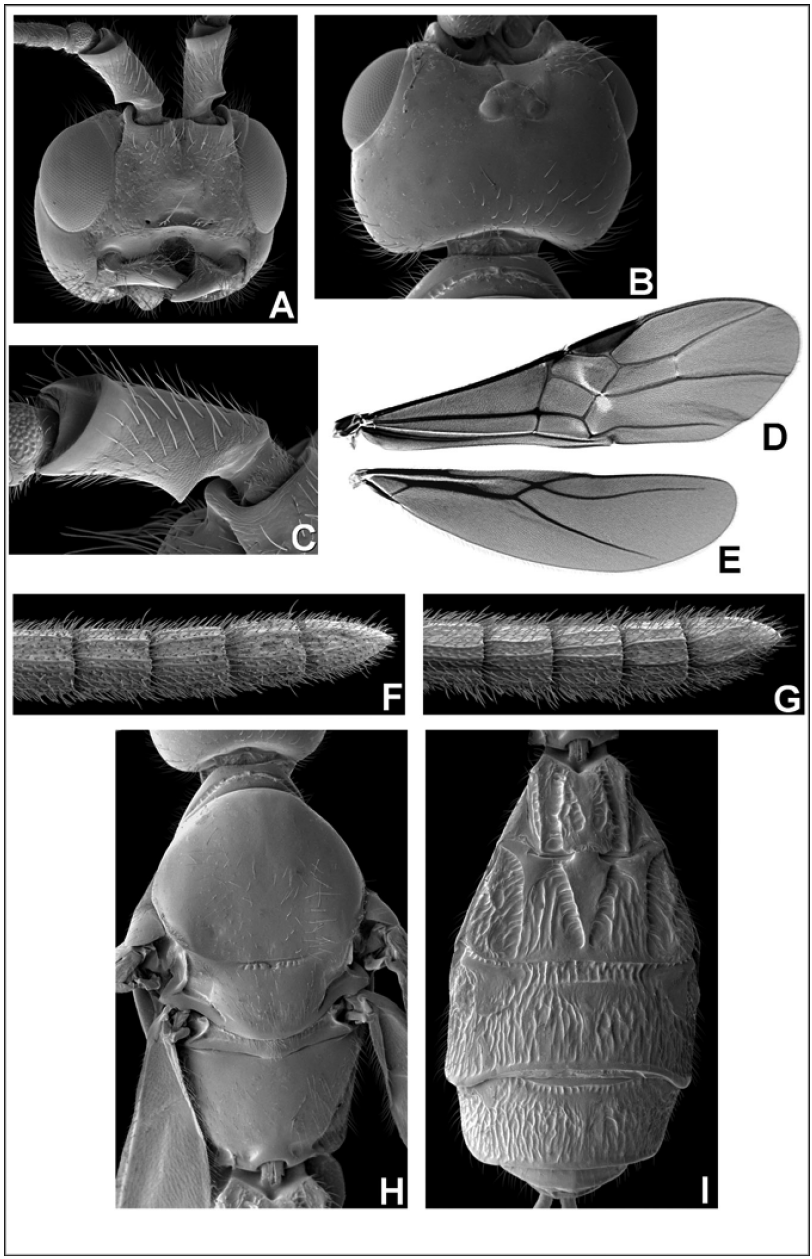


Figure 2. (A-F, H, I) *Atanycolus cappaerti* Marsh and Strazanac, new species: (A) face; (B) vertex and frons; (C) scape, anterior view; (D) fore wing; (E) hind wing; (F) apical flagellomeres; (H) mesosoma, dorsal view; (I) metasoma, dorsal view. (G) *A. hicoriae* Shenefelt, apical flagellomeres.

extending anteriorly from episternal scrobe; propodeum setose laterally, smooth medially, propodeal spiracle usually oval, sometimes round. *Wings* (Fig. 2D, E): fore wing with inner margin of stigma slightly shorter than outer margin, vein r ¼ length of vein 3RSa and slightly shorter than vein 2CU, vein r-m 3/5 length of vein 2RS, vein m-cu nearly 3/5 length of vein 1M; hind wing with vein M+CU about 2/5 length of vein 1M. *Legs*: hind femur 3 times as long as broad; inner apical spur of hind tibia straight. *Metasoma* (Fig, 2I): median plate of first tergum rugulose but often smooth medially, lateral grooves strongly scrobiculate; triangular median plate of second tergum smooth and nearly length of tergum; median width of scrobiculate transverse groove between second and third terga about 1/3 length of third tergum; fourth tergum entirely sculptured; fifth and sixth terga smooth; ovipositor nearly as long as body.

**Male**. Essentially as in female (based on single male specimen available); body length 4 mm; metasomal terga yellowish medially, mesopleuron and mesoscutum marked with brown; antenna with 29 flagellomeres, basal flagellomeres about twice as long as wide; fourth metasomal tergum roughened.

**Holotype female**. Michigan, Fenton, Seven Lakes State Park, 10/12/07, D. Cappaert, ex. EAB galleries (data from single label on specimen). Deposited in National Museum of Natural History, Washington, DC.

**Paratypes**. 12 females, same data as holotype; 10 females, same data as holotype with date of Mar. '08; 12 females, 1 male, same data as holotype with date of Mar./Apr. '08. Deposited in National Museum of Natural History, Washington, DC (12 females, 1 male), Michigan State University, East Lansing, MI (10 females), and West Virginia University, Morgantown, WV (12 females).

**Biology**. Reared from cocoons in emerald ash borer galleries in green ash (*Fraxinus pennsylvanica* Marsh.) and white ash (*F. americana* L.). Host remains of EAB have been observed in association with parasitoid cocoons of *A. cappaerti*, and EAB larvae have been observed with attached parasitoid larvae which subsequently formed cocoons and emerged as adults (D. Cappaert, pers. comm.). Cappaert and McCullough (2009) provide a detailed study of the biology of this species.

**Etymology**. This species is named for David Cappaert, who reared most of the type material, in recognition for his biological studies on this species.

## Acknowledgments

We wish to thank Jim Whitfield and Josephine Rodriguez, Department of Entomology, University of Illinois, for their help during the molecular analysis and in the writing of the results. Kent Hampton, Kansas State University, prepared the SEM micrographs. We also thank three unidentified reviewers for their helpful comments and suggestions. Partial funding was supplied by the USDA Forest Service for which we are grateful.

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