Diagnosing an Overlooked North American Taxon: Biological Observations and Mitochondrial Insights on *Calligrapha suturella* Schaeffer, New Status (Coleoptera, Chrysomelidae)

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ABSTRACT Molecular genetic analyses of populations showing phenotypic variation can provide important insights into the nature and limits of species. This is especially true when interpopulation variation is assessed in a phylogenetic context. The current study applies this approach to evaluate the evolutionary status of the leaf beetle *Calligrapha multipunctata* variety *suturella*, a taxon described from a single locality in New Hampshire, but not further discussed in the literature. Here, we report on the collection of material that greatly extends the geographic range of this taxon and on the analysis of cytochrome oxidase I mitochondrial DNA sequences from these specimens and related *Calligrapha* species. We find that sequences from geographically overlapping populations of *C. m. suturella* and *C. m. bigsbyana* form two distinct, phylogenetically divergent clades that exhibit appreciable differentiation (4.5%) at the nucleotide level. This mtDNA differentiation was also evident in each of three localities where both types were collected together on the same host plant. Our results indicate an absence of gene flow between *C. m. suturella* and *C. m. bigsbyana* and argue for the recognition of *C. m. suturella* as a distinct biological species. Accordingly, we elevate its status to *C. suturella* and provide a detailed diagnosis and description for this new species. Based on an absence of male specimens, a phenotypic similarity to *C. m. bigsbyana*, and the existence of other parthenogenetic *Calligrapha*, we speculate that *C. suturella* may be a parthenogenetic species of hybrid origin.

KEY WORDS insect-plant interactions, molecular systematics, phylogeography, species limits, hybridization

THE LEAF BEETLE GENUS *Calligrapha* Chevrolat, 1837 (Coleoptera: Chrysomelidae: Chrysomelinae) is widely distributed in the Nearctic and Neotropical regions, including 37 species and 4 subspecies in North America north of Mexico (Riley et al. 2002). This genus is especially well studied in northeastern North America, where distribution data exists for 25 species (Brown 1945, Wilcox 1972, Downie and Arnett 1996). These beetles have been included in several faunistic compilations and have been the subject of multiple studies, mostly addressing their host relationships (Hegner 1908, Brown 1945, Mullins 1976, Williams 1989, Forno et al. 1992). To date, however, the species of *Calligrapha* have never been evaluated using molecular genetic approaches, leaving open the possibility that evolutionary relationships and species boundaries do not conform to expectations based on morphological and ecological data.

Calligrapha multipunctata (Say, 1824) is a very widely distributed North American species that has been reported from British Columbia to Nova Scotia in Canada and from Washington to Maine and as far south as Georgia and Arkansas in the United States (Wilcox 1972, Bousquet 1995). Throughout its range, the larvae and adults have been collected exclusively from willows (*Salix* spp.), a host association shared only with *C. verrucosa* (Suffrian, 1858) (Brown 1945, Wilcox 1972). *C. multipunctata* is well characterized morphologically by the presence of more or less developed pale areas on the pronotum, usually as a broad pale band surrounding its lateral and apical margins, and by the lack of the midlateral spot on the elytra present in all other North American *Calligrapha* s. str. (Schaeffer 1933, Wilcox 1972).

Calligrapha multipunctata has been further split into at least three different subspecies or morphological variants. *C. multipunctata* s. str. has paler dark markings on the pronotum and is the most abundant form in the southern range of the species. *C. multipunctata*

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Table 1. Specimens providing mtDNA sequences for the phylogenetic analysis

Taxon	Collection locality	Collection date	Code
Outgroup			
C. elegantula Jacoby 1877	Costa Rica: Alfaro Ruiz, Zarcero	4 June 2000	
Ingroup			
C. apicalis Notman 1919	Canada: Quebec, L'Amiante, Saint-Joseph-de-Coleraine, 45°58′ N 71°21′ W	27 July 2000	
C. philadelphica (Linnaeus 1758)	USA: OH, Pike Co., Big Rock, Jackson Lake	22 July 2001	
C. spiraea (Say 1826)	USA: WV, Greenbrier Co., Tuckahoe Lake	20 July 2001	
C. multipunctata multipunctata (Say 1824)	USA: UT, Cache Co., High Creek	25 July 1992	UT1
C. multipunctata bigsbyana (Kirby 1837)	Canada: Quebec, Charlevoix-Est, Mont-Élie, 47°52′ N 70°29′ W	26 July 2000	QC1
	Canada: Quebec, L'Amiante, Saint-Joseph-de-Coleraine, 45°58′ N 71°21′ W	27 July 2000	QC2
	Canada: Manitoba, Solsgirth, 50°29′ N 100°54′ W, elevation 1770 feet	17 July 2002	MAN1
	USA: ME, Kennebec Co., Sidney, 44°24′ N 69°46′ W, elevation 414 feet	6 June 2002	ME1
	USA: ME, Somerset Co., Moose River, 45°39′ N 70°15′ W	25 July 2000	ME2
	USA: MI, upper peninsula, Luce Co., 46°18′ N 85°37′ W, elevation 768 feet	21 July 2002	MI1
C. suturella Schaeffer 1933 new status	Canada: Quebec, L'Amiante, Saint-Joseph-de-Coleraine, 45°58′ N 71°21′ W	27 July 2000	QC2
	Canada: Quebec, Lac-Saint-Charles, 46°56′ N 71°22′ W	26 July 2000	QC3
	Canada: Manitoba, Franklin, 50°14′ N 99°44′ W, elevation 1649 feet	17 July 2002	MAN2a, MAN2b, MAN2c
	USA: ME, Kennebec Co., Sidney, 44°24′ N 69°46′ W, elevation 414 feet	6 June 2002	ME1
	USA: ME, Somerset Co., Moose River, 45°39′ N 70°15′ W	25 July 2000	ME2
	USA: MI, lower peninsula, Ogemaw Co., Rd I-75, 44°13′ N 84°13′ W, elevation 836 feet	22 July 2002	MI2

bigsbyana (Kirby, 1837) shows the characteristic pale anterior and lateral margins of the pronotum, with the disc and basal margin dark metallic green, and is particularly abundant in the northern United States and Canada. In 1933, *C. multipunctata suturella* Schaeffer was described as a rare local variety of *C. multipunctata* that was differentiated from *C. m. bigsbyana* by a heavier dark maculation on the elytra and a conspicuous darkening of the sutural area. To date, *C. m. suturella* has been known only from Schaeffer's collections in the area of Claremont, NH.

In the course of ongoing research on *Calligrapha* evolution, we have collected specimens on Bebbs willow (*Salix bebbiana*) from a number of geographically widespread localities. While many of these specimens were typical of *C. m. bigsbyana*, others were morphologically consistent with the description of *C. m. suturella.* In this paper, we present a phylogenetic analysis of mitochondrial DNA sequence data from these specimens indicating that *C. m. suturella* represents a distinct biological species. Accordingly, we present a formal redescription of *C. suturella* new status based on our specimens, whose collecting localities considerably extend the known distribution of this taxon. We also discuss scenarios for the evolution of this species, including the possibility of its hybrid origin.

Materials and Methods

Specimens Studied

Material chosen for study (Table 1; Fig. 1) includes (1) all known specimens morphologically corresponding to the type of *C. multipunctata suturella*, (2) *C. m. bigsbyana* from the same or geographically adjacent localities from which *C. m. suturella* specimens were collected, (3) several *Calligrapha* species thought to be closely related to *C. multipunctata* that are easily separable from it morphologically and have different host associations, and (4) a Costa Rican species (*C. elegantula* Jacoby, 1877) used as an outgroup. We were unable to include in the analyses any representatives of *C. verrucosa*, which are presumed to have close affinities with *C. multipunctata.* However, its clear morphological separation from *C. multipunctata* taxa strongly suggests that its inclusion would not change the results and conclusions of the present work.

Molecular Data

For each specimen, whole genomic DNA was extracted using the Dneasy kit (Qiagen, Santa Clarita, CA) following the manufacturer's instructions. One

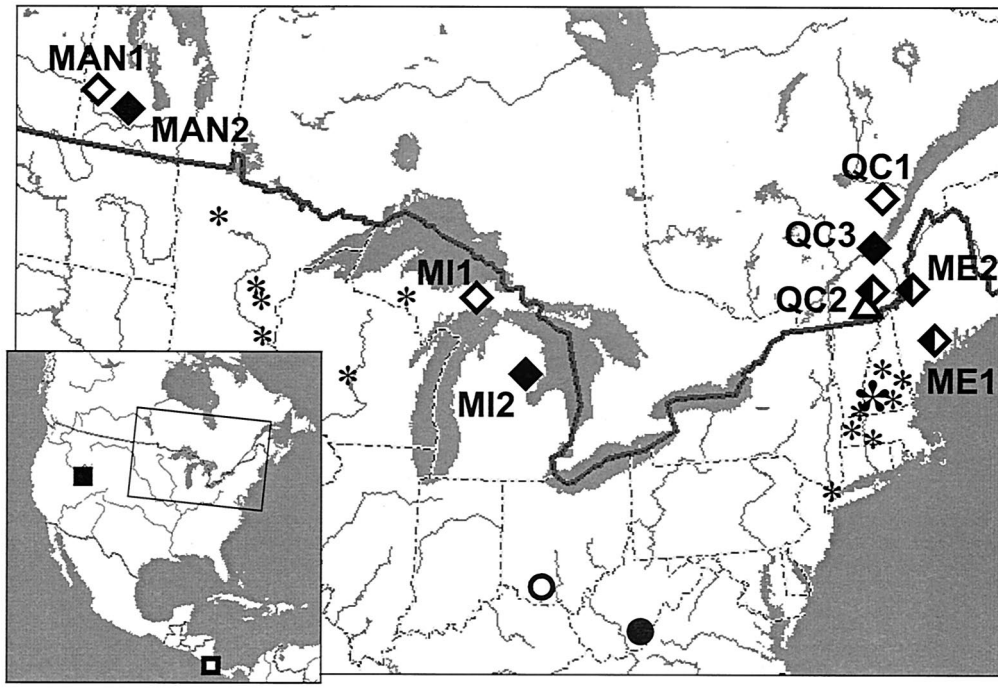

Fig. 1. Collection localities of *Calligrapha* from this study. Localities yielding material for the molecular phylogenetic analysis are indicated by the following symbols: open square, *C. elegantula*; solid square, *C. multipunctata* s. str.; open circle, *C. philadelphica*; solid circle, *C. spiraea*; open triangle, *C. apicalis*; open diamond, *C. multipunctata bigsbyana*; solid diamond, *C. suturella* new status; mixed diamond, *C. m. bigsbyana* + *C. suturella* new status (*C. m. bigsbyana* and *C. suturella* new status collection localities using codes from Table 1). The large asterisk marks the type locality of *C. multipunctata* variety *suturella*, the only known record in the literature. Small asterisks mark additional new localities provided here.

microliter of template DNA was used to amplify the 5′ portion of the cytochrome oxidase subunit I gene (COI) using primers C1-J-2183 (5′-CAACATTTATTTTGATTTTTTGG-3′) and TL2-N-3014 (5′-TCCAATGCACTAATCTGCCATATTA-3′) (Simon et al. 1994). Fifty microliters polymerase chain reaction (PCR) reactions included 2 mM $MgCl_2$ and 10 pmol of each primer and were conducted using the following conditions: 2 min at 96°C followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C. PCR products were purified using the GeneClean II kit (Biosis, La Joya, CA), sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and electrophoresed on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Sequences were edited and assembled using Sequencher v. 4.1.2 software (Gene Codes, Ann Arbor MI). All sequences have been deposited in GenBank under accession numbers AJ575189–AJ575207.

Phylogenetic Analyses

COI sequences were proofread via translation to amino acids and manually aligned using the sequence alignment editor Se-Al v. 2.0a7b (Rambaut and Charleston 2001). Maximum parsimony phylogenetic analysis of the equally weighted data used the branch-and-bound search algorithm implemented in PAUP* v. 4.0b10 (Swofford 2002). Robustness of nodes was assessed by Bremer support indices, using constraint trees generated with TreeRot v. 2 (Sorenson 1999), and by bootstrap analysis using 1,000 pseudo-replicated PAUP* heuristic searches with random addition of taxa (10 repetitions per pseudo-replicate) and TBR branch-swapping.

Results

The aligned COI matrix consisted of 718 nucleotide characters and was free of ambiguities, missing data, or indels. Seventy-four sites were variable, of which 36 were parsimony informative. Two equally parsimonious trees of 85 steps (CI = 0.906; RI = 0.965) were recovered, differing only in the relative position of *C. m. multipunctata* and *C. m. bigsbyana* MAN1 (Fig. 2). *C. m. bigsbyana* sequences formed a monophyletic group, as did *C. m. suturella* sequences. Unexpectedly, the *C. m. suturella* sequences did not group with the other *C. multipunctata* sequences but instead appeared as sister clade to *C. philadelphica* (Linnaeus, 1758). The phylogenetic separation of *C. m. suturella* from other *C. multipunctata* was strongly supported, and these taxa exhibited an average corrected (by Jukes Cantor) sequence divergence of 4.50 ± 0.15%.

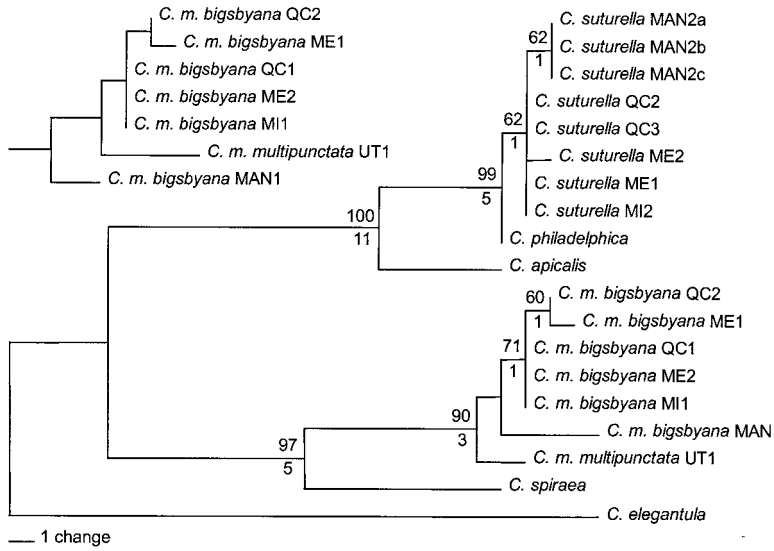

Fig. 2. One of two most parsimonious trees based on *Calligrapha* COI sequences (incongruent region of alternative topology shown in inset). Bootstrap (above branches) and Bremer's support (below branches) values are indicated. Collection localities are identified using codes from Table 1.

Discussion

Species Delineation in *C. multipunctata*

The phylogenetic results of this study argue strongly that *C. multipunctata bigsbyana* and *C. m. suturella* are not conspecific and that they represent neither geographic races nor sympatric morphological polymorphisms. These conclusions are based on the observed mitochondrial monophyly of each group, both of which had been collected from *Salix bebbiana* from geographically interspersed localities across much of northern North America. Maternally inherited, nonrecombining mitochondrial DNA often provides an informative marker for documenting gene flow, which results in the sharing of identical or closely related mitochondrial haplotypes between populations (e.g., Smith, 1992). Conversely, the absence of shared or phylogenetically intermingled haplotypes between *C. m. bigsbyana* and *C. m. suturella* indicates a lack of evolutionarily recent gene flow between them. Even specimens of these two taxa collected from the same plant and at the same time yielded mtDNA sequences from separate clades, indicating that geographic isolation cannot explain our findings and suggesting that reproductive isolating mechanisms prevent gene flow between these taxa even in sympatric, syntopic situations. Furthermore, the sequence divergence between these taxa is greater than that typically observed within individual insect species at mitochondrial loci. Our findings thus suggest that these two taxa belong to separate species under the biological species concept (Mayr 1942).

Our data suggest a close relationship between *C. m. suturella* and *C. philadelphica*, a species that exhibits remarkable variation in its elytral markings (Brown 1945). Given these observations, it might be suspected that *C. m. suturella* represents one of the variants of *C. philadelphica.* However, the observed variation in the latter does not encompass the pigmentation patterns observed in the former. These two species can be consistently distinguished, for example, by the entirely dark pronotum of *C. philadelphica* and the absence of particular elytral markings in *C. m. suturella.* These species also differ in host association, with *C. philadelphica* feeding on various *Cornus* species (Brown 1945). These facts, plus the observed mitochondrial monophyly of *C. m. suturella*, are consistent with the current treatment of these two taxa as separate species.

Redescription of *C. suturella*

Schaeffer (1933), in describing *C. multipunctata* variety *suturella*, made clear his intention to differentiate this taxon from *C. multipunctata bigsbyana.* The name *C. multipunctata suturella*, having been published before 1961 and expressly including the term "variety," can be treated as a subspecific name, therefore becoming available, while its authorship remains to Schaeffer (see articles 10.2, 13, 45.6.4, and 50.3.1, International Commission of Zoological Nomenclature 1999). Following the recommendations of the Code, we consequently propose naming this new morphologically and mitochondrially diagnosed species *Calligrapha suturella* Schaeffer, 1933. Our formal specific description of *C. suturella* based on Schaeffer's type and abundant additional material complements its rather succinct original description.

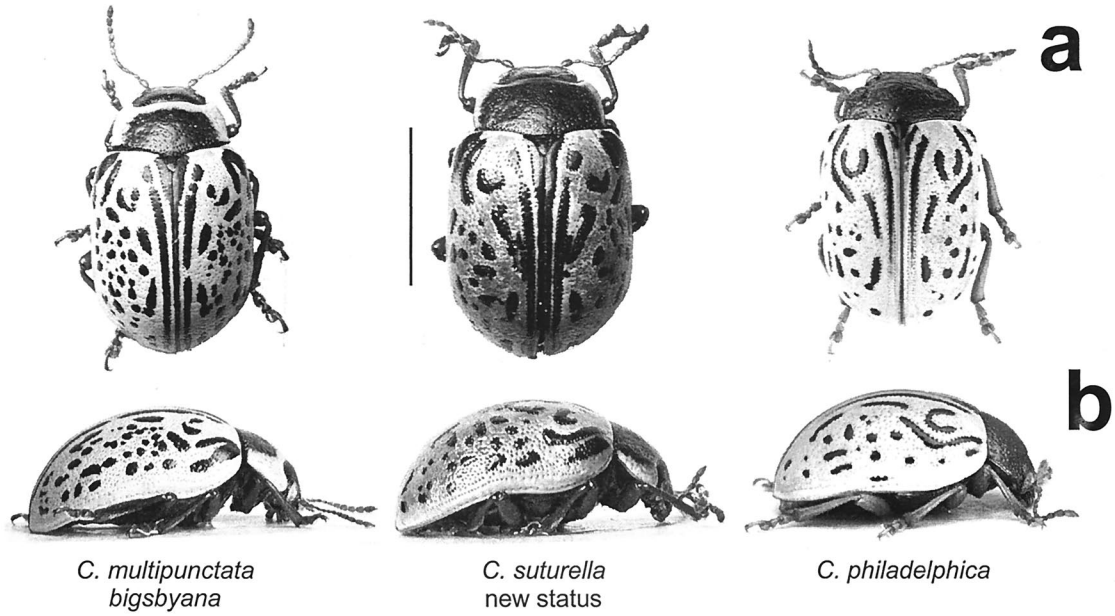

Fig. 3. Dorsal (a) and lateral (b) views of female specimens of *C. multipunctata bigsbyana* (left), *C. suturella* new status (center), and *C. philadelphica* (right). Note differences in pale pronotal band and elytral markings. The scale bar represents 5 mm. The pictures were taken using the Synoptics Auto-Montage Digital 3D Imaging system (Syncroscopy, Cambridge, UK).

Calligrapha suturella Schaeffer, 1933, New Status

Calligrapha multipunctata variety *suturella* Schaeffer, 1933: 478; Wilcox, 1972: 6, 8.

Calligrapha multipunctata suturella Schaeffer. Wilcox, 1975: 65.

Habitus. Dorsally oval in shape; convex in lateral view. Length 8.0–9.2 mm; width 5.2–5.7 mm. (Fig. 3). Elytra creamy yellow; head, pronotum, and numerous elytral markings dark green metallic; antennae, mouthparts, and legs reddish brown.

Head. Black with or without metallic green or bronze luster, with apex of clypeus, labrum, and mouthparts rufous. Strong and relatively dense punctures on a microreticulate surface. Surface largely glabrous except for long white setae on labrum and base of mandibles and fine white pubescence in punctures above eyes, clypeus and antennae. Eyes relatively small (one-eighth head width), dorso-ventrally elongated, and finely faceted. Median frontal suture connected to clypeal suture, forming an inverted Y-shape. Labrum with convergent arcuate sides moderately emarginate apically. Mandibles robust and symmetrical. Last segment of maxillary palpi obliquely truncated at apex with sides subparallel. Antennae 11-segmented, entirely pale reddish brown, extending one-fifth length of elytra. First antennomere oval, about twice as long as wide; second antennomere conical, one-half the length of the first one; third antennomere slender, equal in length to the first one; antennomeres 4–10, subequal in length, progressively thickening, covered with a fine yellowish-white pubescence that is denser in apical segments; last antennomere apically acute and longer than the preceding one.

Pronotum. It is 2.2 times as wide as long; bisinuated in basal and apical margins and with sides regularly curved; wider at base than anteriorly, with maximum width toward the middle. Sparse, heterogeneous punctation present, with coarser punctures on the sides and basally, sometimes elongated and convergent; punctures on disc generally finer, comparable to supraorbital punctures of head; surface between punctures microreticulated, sparsely and minutely punctate. Color black with greenish or bronze reflection; apical angles and lateral margins with a broad yellow band attenuating to dark basal angles; apical margin narrowly margined with a brownish-yellow band continued from pale area in apical angles (see Fig. 3). Setigerous puncture in basal angles with single curved seta projected anteriorly. Scutellum 1.2–1.5 times longer than wide, dark in color and alutaceous.

Elytra. It is 1.3 times longer than wide and convex (ratio height/length: 0.35–0.40). Elytral surface more lustrous than pronotal surface, with confused punctures similar in size to those on pronotal disc; midlateral line of punctures occurring close to and paralleling the epipleura, with another line of punctures in margin between elytron and epipleura; series of punctures encircling dark elytral markings. Color yellowish-white with black or dark metallic green markings as follows (see Figs. 3 and 4): (1) continuous sutural stripe from basal margin of elytra to apical angle and surrounding scutellum, gradually becoming thinner apically; (2) free subsutural stripe, thickened apically, paralleling sutural stripe but not reaching elytral apical angle and broken basally, resulting in an elongated spot close to scutellar area and separated from the rest of the stripe by a distance subequal to its width;

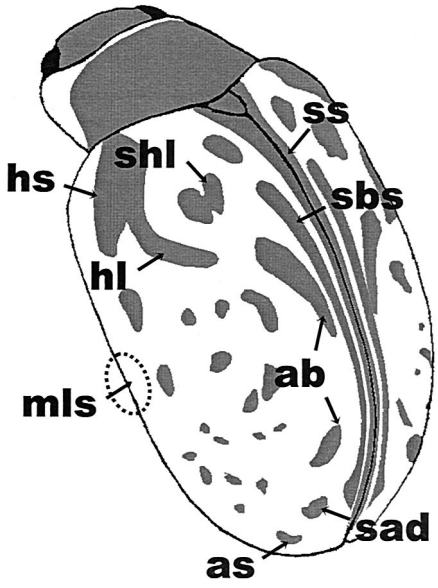

Fig. 4. Schematic representation of the dorsal elytral markings in *C. suturella* new status, including sutural stripe (*ss*), subsutural stripe (*sbs*), arcuate band (*ab*), humeral lunule (*hl*), humeral spot (*hs*), spot enclosed by humeral lunule (*shl*), spot in apical declivity (*sad*), and apical spot (*as*). The position of the midlateral spot (*mls*) of the last elytral interval in other species of *Calligrapha* is also indicated. See text for details.

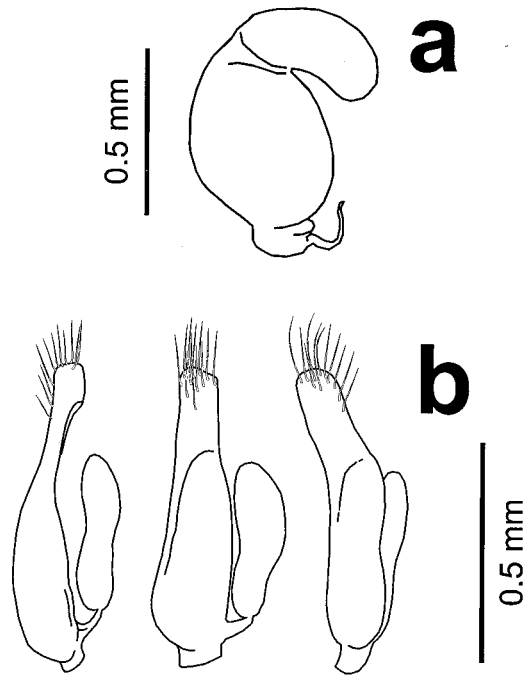

Fig. 5. Schematic drawing of *C. suturella* new status spermatheca (a), and from left to right, right vaginal palp in dorsal, dorso-lateral, and lateral views (b).

(3) humeral lunule continuous or sometimes in the form of two elongated spots describing the contour of the lunule with a small gap or constriction in between; (4) humeral spot large and elongated, attached to base of the humeral lunule or to basal elongated spot; (5) spot enclosed by humeral lunule, subcircular and emarginate anteriorly; (6) arcuate band broadly interrupted after the middle; basal portion strongly convergent with suture posteriorly, and joined to subsutural stripe by areas of dark suffusions; apical portion forming an elongated spot slightly divergent apically from suture; (7) subsutural spot of apical declivity always present, separated from subsutural stripe by a length subequal to the local width of the stripe; (8) apical spot on elytra subequal to subsutural spot in size and sometimes confluent with it; and (9) additional markings on disc consisting of 14–20 small, irregular, sometimes confluent spots without any clear pattern. Elytral epipleura as pale as palest parts of the elytra. Hind wings present and red in color.

Venter. Dark metallic green. Epimera of pronotum impunctate and microsculptured, with some ridges and notches basally. Prosternum rugose-punctate with relatively long whitish setae; apical margin rounded. Meso- and metasternum without modifications. Metaepimeron with coarse punctures. Metathorax and abdominal segments with a sparser and finer punctation that is almost obsolete medially, and with sparse, fine and short transparent pubescence. Legs light brown or reddish, except for very dark brown coxae with a hint of metallic sheen; presenting some short, evenly distributed, golden setae. Tibiae gradually thickening to apex, meso-, and metatibiae with a longitudinal anterior notch presenting an anterior bead and disappearing toward apex; apical enlargement of tibiae coarsely and densely punctured and densely covered by a short golden pubescence. Tarsi inserted apically on tibiae; second tarsomere one-half as long as and narrower than first tarsomere, both covered ventrally by dense pubescence; third tarsomere 1.5 times as wide as second, slightly emarginate anteriorly, and with longer and thicker pubescence; last tarsal segment without modifications and with simple, divergent claws. Spermatheca and vaginal palpi present and similar to those of congeneric species (Fig. 5).

Material Examined. *Type* one female, USA, NH, Claremont, Coll. Schaeffer. *Material used in genetic analyses:* one female, Canada, Quebec, L'Amiante, Saint-Joseph-de-Coleraine (45°58′N 71°21′W), 27 July 2000, on *Salix bebbiana*, D. J. Funk (QC2); one female, Canada, Quebec, Lac-Saint-Charles (46°56′N 71°22′W), 26 July 2000, on *Salix bebbiana*, D. J. Funk (QC3); three females, Canada, Manitoba, Franklin (50°14′N 99°44′W, 1649 feet), 17 July 2002, on *Salix bebbiana*, D. J. Funk (MAN2a, MAN2b and MAN2c); one female, MI, lower peninsula, Ogemaw Co., Rt. I-75 (44°13′N 84°13′W, 836 feet), 22 July 2002, on *Salix bebbiana*, D. J. Funk (MI2); one female, ME, Kennebec Co., Sidney (44°24′N 69°46′W, 414 feet), 6 June 2002, on *Salix bebbiana*, D. J. Funk (ME1); one female, ME, Somerset Co., Moose River (45°39′N 70°15′W), 25

July 2000, on *Salix bebbiana*, D. J. Funk (ME2). *Other material:* one female, ME, Kennebec Co., Sidney (44°24′N 69°46′W, 414 feet), 20 June 2003, on *Salix bebbiana*, D. J. Funk; 2 females, ME, Cumberland Co., Portland (43°39′N 70°21′W, 105 feet), 18 June 2003, on *Salix bebbiana,* D. J. Funk; 1 female, MA, Berkshire Co., Hinsdale, 21 August 1898; one female, MA, Hampden Co., Ludlow, 22 June 1902; one female, MA, Hampden Co., Wilbraham, J. O. Martin; one female, MA; one female, MI, Baraga Co., 10 miles W Three Lakes, 7 June 1982, David R. Smith; one female, MI, Ag. Coll. Michigan, 1891, C. F. B., F. Knab coll. 1918; one female, MN, St. Anthony Pk., 9 June 1907; one female, MN, Aitkin Co., 3 miles S McGrath, 3 July 1984, Downie and Wappes; one female, MN, Becker Co., Itasca St. Pk. Area, 28/29 June 1984, Downie and Wappes; two females, MN, Kanabec Co., Mora, 25 August 1907, R. A. Vickery; four females, NH, Hillborough Co., Manchester, 16 June, 25 June and 5 July 1932, W. S. Abbott; three females, NH, Strafford Co., Milton, 3 July 1897, F. Knab coll. 1918; one female, NH, Squam Lake, F. Knab coll. 1918; one female, NY, Van Cortland Park, Charles Schaeffer coll.; two females, VT, Windham Co., Brattleboro, spring 1894, F. Knab coll. 1918; one female, WI, Wood Co., Cranmoor, 26 August 1909, C. W. Hooker.

Schaeffer's type of *C. suturella* and most of the material examined are deposited at the U.S. National Museum of Natural History (NMNH). The specimens collected by DJF and used for molecular analyses are deposited in the Natural History Museum (London, UK; MANa1, MANa2, and MANa3), in the U.S. NMNH (Washington, DC; ME1, ME2 [specimen in Fig. 3], MI2, and QC3), and in the personal collection of JGZ (QC2).

Species Variability. Some morphological variation exists among *C. suturella* specimens. This was apparent in the sutural, subsutural, and arcuate bands (*ss*, *sbs*, and *ab*, respectively, Fig. 4), which may appear to be either completely free or confluent owing to dark suffusions. Also, the humeral lunule and the humeral spot (*hl* and *hs* in Fig. 4) appear detached in some specimens. In three *C. suturella* specimens (ME1 and the series from Vermont), the front pale margin of the pronotum is broader, resembling that of *C. m. bigsbyana*, but the elytral markings show the band confluence and reduced number of elytral spots typical of *C. suturella.*

Differential Diagnosis. Specimens of *C. suturella* are somewhat larger (8.0–9.2 mm) than those of *C. multipunctata bigsbyana* (6.5–8.0 mm on average; Wilcox, 1972) with heavier elytral markings and convergence of the sutural and subsutural bands or the subsutural and arcuate bands (see Fig. 3). Also, *C. m. bigsbyana* has more numerous elytral markings, typically with >25 small spots compared with the 14–20 on the studied specimens of *C. suturella.* However, the most diagnostic characteristics are those of the pale pronotal band, which is broad and conspicuous along its entire length in *C. multipunctata bigsbyana* (which sometimes also exhibits paler areas in the disc of the pronotum), but is thinner and darker along the anterior margin in *C. suturella.* The anterior portion of this band can be very thin and darkened in some specimens, making it appear that the pronotum is only pale in the lateral margins or in the apical angles. This characteristic and the heavier elytral markings are typical of another species, *C. apicalis* Notman, as was noted in the original description of *C. suturella.* However, these species are easily distinguished by the midlateral spot that is present in *C. apicalis* but always absent in *C. suturella.* These taxa also differ in host plant association, with *C. suturella* using *Salix* spp. and *C. apicalis* only reported from *Alnus incana americana* (Brown 1945). As discussed above, a possible subordination of *C. suturella* to *C. philadelphica* based on the genetic data are incompatible with the consistent morphological and ecological differentiation of these taxa. The midlateral spot and entirely dark pronotum of *C. philadelphica* (see Fig. 3) and its association with *Cornus* spp. clearly distinguish it from *C. suturella.*

Distribution and Abundance. The original description of *C. suturella* was entirely based on specimens collected from a local area in New Hampshire (Schaeffer 1933). Before the current study, no further literature records of this taxon existed, possibly because of confusion with other *Calligrapha* taxa, specifically *C. multipunctata bigsbyana* and *C. apicalis.* Among 96 *Calligrapha* specimens collected on *Salix Bebbiana* in recent years by DJF, 13% proved to be *C. suturella* and 87% were *C. m. bigsbyana.* Based on these limited initial data, it seems that *C. suturella* may often be less common than *C. m. bigsybyana* but is not an extremely rare species. These specimens and others from the U.S. NMNH greatly extend the known geographic distribution of *C. suturella* by adding new collection localities in Maine, Manitoba, Massachusetts, Michigan, Minnesota, New York, Quebec, Vermont, and Wisconsin (Fig. 1). Further fieldwork and the inspection of additional American entomological collections will be required to firmly establish the geographical limits of this widely distributed species.

Speculations on the Evolutionary Origin of *C. suturella*. The description of *C. suturella* as a local variety of *C. multipunctata* was likely motivated by the striking resemblance of these taxa with respect to several characters assumed to be apomorphic for *C. multipunctata* (Fig. 3). First, *C. suturella* possesses a pale band in the anterior and lateral margins of the pronotum, a character only present in *C. m. bigsbyana* among the North American *Calligrapha* s. str. (Wilcox 1972). Second, the midlateral spot on the last interval of the elytra is present in all Nearctic *Calligrapha* except *C. suturella*, *C. multipunctata*, and the closely related *C. verrucosa* (Fig. 4). Third, *C. suturella* and *C. multipunctata* exhibit similar arrangements of spots and other markings on the elytra, characters that are useful for species identification in this genus. Fourth, *C. multipunctata* and *C. suturella* share the same host plant, a highly valuable taxonomic character in *Calligrapha*, which includes many apparently monophagous species (Brown 1945, Robertson 1966, Wilcox 1972). The association of these beetle species with

Salix spp. is shared only with *C. verrucosa* among North American *Calligrapha.*

The considerable morphological and ecological similarity of *C. m. bigsbyana* and *C. suturella* are intriguing in view of their considerable phylogenetic separation and divergence in mitochondrial DNA. Such patterns would seem to indicate the convergent evolution of many traits in each of two separate beetle lineages. A possible alternative scenario, however, could explain these observations in terms of single evolutionary origins for homologously shared phenotypic traits. In various animal taxa, it has been shown that parthenogenetic, polyploid species have originated by interspecific hybridization between two sexual, diploid species. For example, parthenogenesis and polyploidy are both widespread in the weevils (Curculionoidea), and this scenario seems to explain the origin of particular species in the weevil genus *Aramigus* (Normark and Lanteri 1998). This mechanism could explain the patterns of *Calligrapha* trait variation described above under the following conditions: (1) the interspecific hybridization involved two evolutionarily divergent parental species, (2) this hybridization was unidirectional, such that males of one parental species mated with females of the other but not vice versa, and (3) many paternal alleles for phenotypic traits were dominant.

These assumptions seem plausible given findings from other systems. First, mtDNA and Y-chromosome data suggest that hybrid speciation in animals usually involves unidirectional hybridization (Wirtz 1999). Second, a mechanism for uniparental character dominance has been suggested for certain hybrid weevil lineages. In these insects, polyploidization seems to involve the accumulation of multiple haploid genomes from the paternal parent of the hybrid lineage (Saura et al. 1993, Normark and Lanteri 1998). These polyploid hybrid species thus exhibit a much greater phenotypic resemblance to the paternal than the maternal species. They are also parthenogenetic.

Although parthenogenesis is rather rare in chrysomelids (Cox 1996, Jolivet and Verma 2002), the biology of *Calligrapha* and of *C. suturella* suggests the plausibility of the scenario described above. A number of *Calligrapha* species from northeastern North America are known to be parthenogenetic tetraploids, exhibiting obligate thelytoky (Brown 1945, Robertson 1966). Thus, it is particularly noteworthy that all of the 35 *C. suturella* specimens identified to date have been females. The possibility that *C. suturella* is obligately parthenogenetic would further explain how it is able to locally coexist on the same host plant (*Salix bebbiana*) with *C. m. bigsbyana* without any gene flow between them. *Calligrapha suturella* specimens are also larger than closely related diploid species, a phenomenon often observed for polyploid taxa. Under our hybrid speciation scenario, a polyploid, parthenogenetic *C. suturella* would be the result of matings between male *C. m. bigsbyana* and females of *C. philadelphica* (a sympatric, sexually reproducing diploid species) or a related species from this lineage. An evolutionarily recent hybrid speciation event of this kind would also explain the high similarity between *C. suturella* and *C. philadelphica* in mtDNA. Presently, this scenario remains quite hypothetical, however. Future research on ploidy, nuclear genes, and reproductive biology will help to rigorously evaluate the origin of *C. suturella.*

Conclusions. This study illustrates the value of population-level sampling and molecular systematic tools for addressing the important systematic issues of species diagnosis and the establishment of species limits. Without the insights provided by such phylogeographic data, taxa such as *C. suturella* will doubtless continue to be overlooked. Using such approaches to distinguish within-species phenotypic polymorphism from the traits diagnostic of separate biological species will be increasingly critical to improved assessments of biodiversity in many taxa.

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