Wing Morphometry Helps Diagnose Cryptic Species and Resurrect *Mindarus pinicolus* (Hemiptera: Aphididae)

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ABSTRACT Wing venation, two-dimensional and with easily recognized reference points at vein junctions, presents an opportunity for the development of automated insect identification. Using a suite of continuous characters, I investigated the use of wing morphometry for computerized insect identification of cryptic species of the aphid genus *Mindarus*. A priori groups were determined using cytochrome oxidase 1 DNA barcodes. Discriminant function analysis of 24 wing measurements consistently grouped individuals of unknown taxonomic affinity with the correct a priori groups. The results suggest that diagnostic signal is present in wing morphometry, but the signal is considerably stronger with the addition of morphometry from other aphid appendages, namely, 10 leg and antennal segments. Almost all *Mindarus* collected in eastern North America have been determined as the balsam twig aphid, *Mindarus abietinus* Koch (Hemiptera: Aphididae), but molecular diagnostics reveals that the Palearctic species is not present in the Western Hemisphere. *Schizoneura pinicola* Thomas has been considered a North American synonym of *M. abietinus*. Morphometric discriminant function analysis suggests that the *Abies*-feeding eastern North American population is *M. pinicolus*. The species is here reinstated with a new combination and redescribed.

KEY WORDS *Mindarus abietinus*, discriminant functions, DNA barcodes

High-powered computing and artificial intelligence offer new hopes for automated insect identification and various techniques that target insect wing images have been developed (Baylac et al. 2003, Steinhage et al. 2007, Bhanu et al. 2008). Two-dimensional images of insect wings are ready-made for analysis (Tofilski 2007).

Aphids are a taxonomically complex and morphologically reduced group of insects, one that has required the use of morphometric techniques even for basic taxonomic diagnoses. Many species-level aphid dichotomous keys require a significant amount of measuring and computing of ratios (Eastop 1971, Corpuz-Raros and Cook 1974, Robinson 1985, Sorensen 1994). Much insect morphometrics was pioneered using aphid models (Sokal 1952, 1962), and numerous species have been described based on morphometry (Blackman 1987, Sorensen 1994, Watson et al. 1999, Lozier et al. 2008).

Discriminant function analysis is a multivariate technique that maximizes the morphometric distance between predetermined groups (Pimentel 1992). As performed by the software program SYSTAT 10 (SPSS Inc., Chicago, IL), discriminant function analysis establishes a centroid for each a priori group of individuals and then calculates the Mahalanobis distance between each individual and the various centroids. Individuals are then classed a posteriori into the group whose centroid is closest. There is a lack of full independence in the results because the a posteriori individuals belonged to the a priori groups. An independent test of the discriminant functions is to add individuals as their own separate classes, that is, not belonging to any of the a priori groups. These "unknowns" are added to the analysis to ascertain their morphometric proximity to the a priori groups and as a test of the accuracy of the method. Discriminant functions have been used to discriminate between aphid species (Brown and Blackman 1994), populations of the same species (Damsteegt and Voegtlin 1990), between holocyclic and anholocyclic aphids of the same species (Hand 1986), and between fundatrices and apterous viviparae of the same species (Favret et al. 2004). Favret and Voegtlin (2004a) attributed type specimens of several dubious species to other species, thereby establishing synonymies.

Recent molecular evidence suggests the aphid genus *Mindarus* harbors several cryptic species (Favret and Nielsen 2008; unpublished data). I wanted to test whether wing morphometry alone could diagnose the same species that were recognized with molecular data. Measurements of individual specimens were added to a discriminant function analysis to assess their morphometric proximity to a priori groupings

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Table 1. List of specimens used in molecular and morphometric analyses

INHS catalog	Species	Country	State or province	County	Latitude	Longitude	Collector(s)	Date	Host	GenBank no.
18,301	Appalachian	U.S.A.	North Carolina	Haywood	35.58	−83.07	C. Favret	05/31/2003	*Abies fraseri*	FJ668253
61,606	Appalachian	U.S.A.	North Carolina	Haywood	35.58	−83.07	C. Favret	05/18/2004	*A. fraseri*	
61,654	Appalachian	U.S.A.	North Carolina	Haywood	35.58	−83.07	C. Favret	05/18/2004	*A. fraseri*	FJ668251
411,801	Appalachian	Canada	Quebec		47.78	−70.23	C. Favret, S. Favret	06/21/2006	*Abies balsamea*	FJ668254
411,803	Appalachian	U.S.A.	Vermont	Washington	44.08	−72.86	C. Favret, S. Favret	06/19/2006	*A. balsamea*	FJ668259
411,804	Appalachian	U.S.A.	New York	St. Lawrence	44.13	−74.63	C. Favret, S. Favret	06/18/2006	*A. balsamea*	FJ668256
411,806*	Appalachian	U.S.A.	North Carolina	Haywood	35.58	−83.07	C. Favret, S. Favret	06/14/2006	*A. fraseri*	FJ668252
411,808	Appalachian	U.S.A.	Maine	Aroostook	45.66	−68.28	C. Favret, S. Favret	06/20/2006	*A. balsamea*	FJ668264
411,809	Appalachian	U.S.A.	Maine	Aroostook	46.66	−68.24	C. Favret, S. Favret	06/20/2006	*A. balsamea*	FJ668258
411,813	Appalachian	Canada	Quebec		47.51	−70.51	C. Favret, S. Favret	06/21/2006	*A. balsamea*	FJ668257
411,816	Appalachian	U.S.A.	Vermont	Addison	44.00	−73.02	C. Favret, S. Favret	06/18/2006	*A. balsamea*	FJ668263
411,818	Appalachian	U.S.A.	Maine	Aroostook	46.24	−68.34	C. Favret, S. Favret	06/20/2006	*A. balsamea*	FJ668265
411,819	Appalachian	Canada	Quebec		47.59	−68.72	C. Favret, S. Favret	06/21/2006	*A. balsamea*	FJ668261
411,821	Appalachian	U.S.A.	New York	Oneida	43.59	−75.12	C. Favret, S. Favret	06/17/2006	*A. balsamea*	FJ668262
411,822	Appalachian	U.S.A.	New Hampshire	Grafton	44.10	−71.84	C. Favret, S. Favret	06/19/2006	*A. balsamea*	FJ668266
411,829	Appalachian	U.S.A.	Maine	Aroostook	47.29	−68.50	C. Favret, S. Favret	06/21/2006	*A. balsamea*	FJ668255
411,830	Appalachian	U.S.A.	Vermont	Addison	43.94	−72.95	C. Favret, S. Favret	06/19/2006	*A. balsamea*	FJ668260
96,471	*M. abietinus*	Italy	Tuscany	Firenze			A. Binazzi	06/07/2005	*Abies alba*	FJ668245
411,860	*M. abietinus*	Denmark	Midtjylland	Silkeborg			C. Nielsen	06/30/2006	*Abies* sp.	FJ668244
96,457	*M. kinseyi*	U.S.A.	Washington	Whatcom	48.84	−122.27	C. Favret	06/18/2005	*Abies grandis*	FJ668249
96,459	*M. kinseyi*	U.S.A.	Washington	Stevens	48.54	−117.61	C. Favret	06/21/2005	*A. grandis*	FJ668246
96,463	*M. kinseyi*	U.S.A.	Washington	Ferry	48.64	−118.44	C. Favret	06/21/2005	(none, in flight)	FJ668247
96,530	*M. kinseyi*	U.S.A.	Washington	Cowlitz	46.30	−122.82	C. Favret	06/12/2005	*A. grandis*	FJ668248
96,541	*M. kinseyi*	U.S.A.	California	El Dorado	38.74	−120.73	C. Favret	06/06/2005	*Abies concolor*	FJ668250
179,752	*M. kinseyi*	U.S.A.	California	El Dorado	38.74	−120.73	C. Favret	06/06/2005	*A. concolor*	
179,861*	*M. kinseyi*	U.S.A.	Washington	Cowlitz	46.30	−122.82	C. Favret	06/12/2005	*A. grandis*	
411,854	Rockies 1	U.S.A.	Utah	San Juan	38.42	−109.25	C. Favret	06/30/2007	*A. lasiocarpa*	FJ668268
411,856	Rockies 1	U.S.A.	Wyoming	Johnson	44.00	−107.03	C. Favret	07/04/2007	*A. lasiocarpa*	FJ668271
411,864	Rockies 1	U.S.A.	Arizona	Coconino	36.38	−112.11	C. Favret	06/28/2007	*Abies lasiocarpa*	FJ668270
411,871	Rockies 1	U.S.A.	Montana	Gallatin	45.51	−111.11	C. Favret	07/06/2007	*A. lasiocarpa*	FJ668269
411,873	Rockies 1	U.S.A.	Montana	Beaverhead	45.69	−113.93	C. Favret	07/07/2007	*A. lasiocarpa*	FJ668272
411,876	Rockies 1	U.S.A.	Colorado	Clear Creek	40.51	−105.90	C. Favret	07/03/2007	*A. lasiocarpa*	FJ668274
411,901	Rockies 1	U.S.A.	Oregon	Linn	44.42	−121.87	C. Favret	07/11/2007	*A. lasiocarpa*	FJ668267
411,907*	Rockies 1	U.S.A.	New Mexico	Los Alamos	35.90	−106.41	C. Favret, K.E.F. Favret	06/23/2007	*A. concolor*	FJ668277
411,910	Rockies 1	U.S.A.	New Mexico	Los Alamos	35.89	−106.40	C. Favret, K.E.F. Favret	06/23/2007	*A. lasiocarpa*	FJ668279
411,914	Rockies 1	U.S.A.	New Mexico	Cibola	35.25	−107.60	C. Favret	06/26/2007	*A. lasiocarpa*	FJ668278
411,922	Rockies 1	U.S.A.	Colorado	San Juan	37.90	−107.72	C. Favret	07/01/2007	*A. lasiocarpa*	FJ668273
411,924	Rockies 1	U.S.A.	New Mexico	Los Alamos	35.89	−106.40	C. Favret, K.E.F. Favret	06/23/2007	*A. lasiocarpa*	FJ668275
411,925	Rockies 1	U.S.A.	Wyoming	Carbon	41.00	−106.98	C. Favret	07/03/2007	*A. lasiocarpa*	FJ668276
179,776	Rockies 2	U.S.A.	Illinois	Champaign	40.10	−88.23	C. Favret	05/13/2005	*A. concolor*	
411,853	Rockies 2	U.S.A.	Arizona	Coconino	36.42	−112.09	C. Favret	06/28/2007	*A. concolor*	FJ668280
411,862	Rockies 2	U.S.A.	New Mexico	Los Alamos	35.87	−106.35	C. Favret, K.E.F. Favret	06/23/2007	*A. concolor*	FJ668287
411,866	Rockies 2	U.S.A.	Colorado	Ouray	38.00	−107.66	C. Favret	07/01/2007	*A. concolor*	FJ668289
411,875	Rockies 2	U.S.A.	Colorado	Custer	38.26	−105.66	C. Favret	07/02/2007	*A. concolor*	FJ668283
411,886	Rockies 2	U.S.A.	Arizona	Coconino	36.64	−112.17	C. Favret	06/27/2007	*A. concolor*	FJ668281
411,900	Rockies 2	U.S.A.	New Mexico	Torrance	34.68	−106.42	C. Favret, K.E.F. Favret	06/24/2007	*A. concolor*	FJ668282
411,909	Rockies 2	U.S.A.	New Mexico	Los Alamos	35.83	−106.38	C. Favret, K.E.F. Favret	06/23/2007	*A. concolor*	FJ668286
411,911*	Rockies 2	U.S.A.	New Mexico	Sandoval	35.86	−106.63	C. Favret, K.E.F. Favret	06/23/2007	*A. concolor*	FJ668284
411,913	Rockies 2	U.S.A.	New Mexico	Sandoval	35.85	−106.43	C. Favret, K.E.F. Favret	06/23/2007	*A. concolor*	FJ668285
411,918	Rockies 2	U.S.A.	New Mexico	Sandoval	35.86	−106.43	C. Favret, K.E.F. Favret	06/23/2007	*A. concolor*	FJ668288
199,996*	*S. pinicola*	U.S.A.	Illinois	Jackson			C. Thomas	04/20/1878	*Pinus strobus*	

Asterisks (*) denote specimens added singly to the morphometric analyses.

based on molecular data. Along with these specimens of known taxonomic affinity, I also tested the morphometric affinity of the holotype of *Schizoneura pinicola* Thomas (1879), a North American junior synonym (Patch 1910) of the European-described *Mindarus abietinus* Koch (1857).

The genus *Mindarus* is an excellent model for testing the validity of wing vein morphometric discrimination because many of the species are cryptic, and indeed as yet undescribed. Species of *Mindarus* are pestiferous in Christmas tree farms (Nettleton and Hain 1982, Kleintjes et al. 1999, Fondren and McCullough 2003) and nurseries (Ehler and Kinsey 1995). The balsam twig aphid causes unsightly needle curl diminishing the value of harvested trees in the eastern and Midwestern regions of the United States. The North Carolina Christmas tree industry alone has an estimated $100 million or more in annual cash receipts, with pesticide applications to control the balsam twig aphid typical the last 2 yr before trees are harvested (Sidebottom 2008).

Materials and Methods

Collections were made of *Mindarus* on *Abies*, true firs, throughout the western and eastern U.S. mountain states and some Canadian provinces in the late springs and summers of 2003 through 2007. Fresh specimens of *M. abietinus* in Italy and Denmark also were obtained (Table 1). QIAGEN kits were used to extract nondestructively the DNA from single alate individuals in numerous colonies (Favret 2005). The intact and cleared cuticles from the specimens were then mounted to microscope slides in Canada balsam for morphological analysis. All of these fresh specimens are deposited in the insect collection of the Illinois Natural History Survey, Champaign, IL (INHS). The holotype of *S. pinicola* was borrowed from the INHS.

Partial cytochrome oxidase 1 (CO1) DNA sequences were acquired using primers and standard techniques described by Favret and Voegtlin (2004b). Forward and reverse sequences were combined, and edited sequences aligned, using Sequencher 4.7 soft-

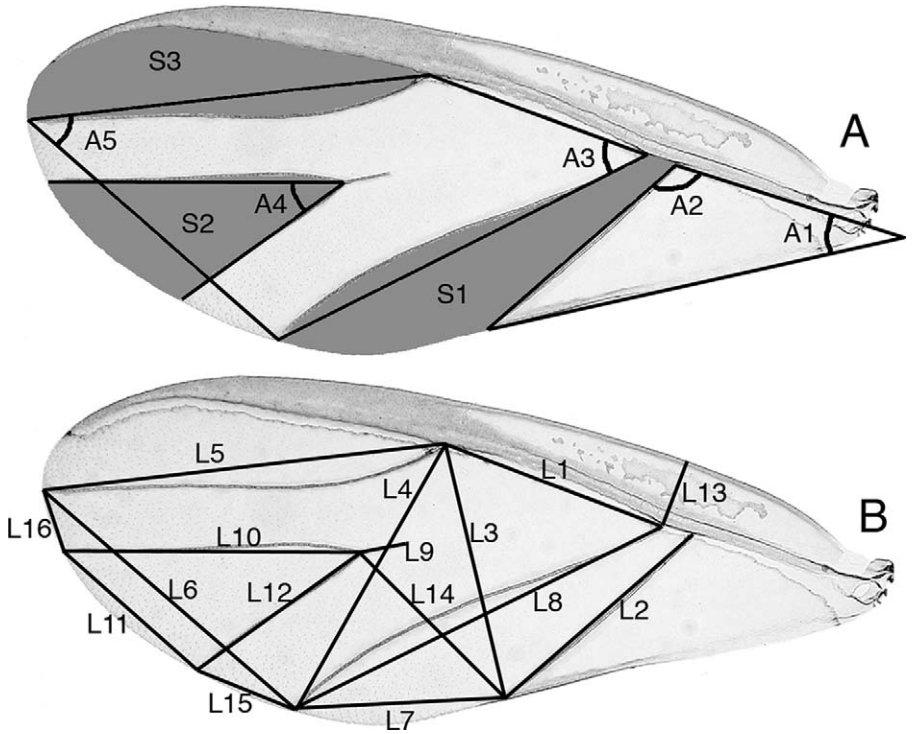

Fig. 1. Diagrams of wing morphometry. (A) Measured wing cell surface areas (S1–3, in shaded areas) and angles (A1–5, between dark lines). (B) Length measures (L1–16).

ware (Gene Codes Corporation, Ann Arbor, MI). A parsimony-based phylogeny of the *Mindarus* CO1 sequences was estimated using PAUP*(4b10) software (Swofford 2002), with 2,000 heuristic bootstrap replicates, each with 10 random addition replicates, the Multrees option turned off, by using tree bisection and reconnection branch swapping. Uncorrected P distances were calculated with PAUP*. TCS software (Clement et al. 2000) was used to perform nested clade analysis to estimate groupings of terminal branches.

A suite of wing measurements was chosen based on ease of consistent measuring. For example, wing cell surface areas were measured for defined cells with clear borders, and length measures were used only if end points could be located clearly. Sixteen length measures, five wing vein angles, and three cell surface areas were measured for a total of 24 wing morphometrics (Fig. 1). In addition, the lengths of the forefemur, foretibia, foretarsus 2, metafemur, metatibia, metatarsus 2, and flagellomeres 3–6 also were measured, and the number of sensoria on the third antennal segment counted. When the specimens were intact, measurements were made of both the right and left sides of the aphid and the ensemble of each set of left- and right-hand measurements treated as separate specimens. In this way, I was able to almost double the number of data points for analysis. Parts of legs or antennae were missing from one side of 11 of the specimens. In these cases, I either used measurements of the appendage from the other side of the aphid or prorated the length using the ratio of the intact parts of the appendage on both sides to calculate the missing appendage segment. Measurements were made using AxioVision 4.6 imaging and measuring software (Carl Zeiss, Göttingen, Germany) and an Axio Imager M1 microscope (Carl Zeiss) and were recorded in micrometers.

A priori groups defined by the molecular analyses were used in linear discriminant functions analyses (DFA) by using SYSTAT 10 software with equally weighted variables and the default matrix inversion tolerance of 0.001. Four specimens without molecular data, but of known taxonomic identity, were included to supplement the numbers in the analysis (Table 1). A single individual (often resulting in two sets of measurements as described above) from each of the four putative species was randomly selected and submitted to the analysis as an unknown individual. The proximity of these individuals to the a priori groups in DFA would determine their taxonomic affinity. Separate analyses also were conducted with the *S. pinicola* holotype. Because the holotype specimen is missing both hind legs, DFAs that included the holotype omitted hind leg lengths. In all, five DFAs were run: 1) all specimens using only wing data; 2) all specimens except the holotype using wing and appendage data; 3) all specimens except the holotype using only appendage data; 4) all specimens using wing, foreleg, and antennal data; and 5) all specimens using only foreleg and antennal data (Table 2).

Table 2. Summary of DFA, including the number of misclassed individuals within the four groups

No.	Included data		Type included?	Misclassed individuals	Related figure
	Wing	Appendage			
1	Yes	No	Yes	3	4
2	Yes	Yes	No	0	3
3	No	Yes	No	13	
4	Yes	Yes, no hing leg	Yes	0	5
5	No	Yes, no hind leg	Yes	20	

Results

Analysis of the CO1 sequences revealed five well-supported terminal clades (Fig. 2): *M. abietinus* from Europe, *Mindarus kinseyi* Voegtlin (1995) from the U.S. Pacific Northwest, and three undescribed species, two from the Rocky Mountains and one from the Appalachians. All five had bootstrap support values of 98 or higher and distances of 3.84% or higher among them (Fig. 2). Only the clade of *M. kinseyi* showed any partitioning, with two well-supported smaller clades (bootstrap support of 97 and 99 and distance of 2%). *M. abietinus* from Europe was clearly distinct from all of the North American collections, with distances of 5.88% or higher. Nested clade analysis with 95% statistical confidence recovered all five clades as distinct and separate networks.

The four putative species showed mean differences in 30 of the 35 morphometrics (Table 3). Appendage morphometrics were all correlated for size, with the two Rockies species larger than the other two species for every measure except the length of the metafemur. *M. kinseyi* was always the next largest, and the Appalachian species was the smallest for every measure (again, except the metafemur). Wing morphometrics did not show the same correlation as appendage lengths, however. Although 11 wing morphometrics had the same size pattern as the appendages (Rockies 1 or 2 largest and second largest, followed by *M. kinseyi,* followed by Appalachian), nine wing measurements exhibited different size patterns (Table 3).

The four North American putative species were used as a priori groups in various discriminant functions analyses. The analysis that included all wing and appendage morphometry had the clearest discrimination (Fig. 3) with no individual being misclassed by DFA (Table 2). The first three factors exhibited the strongest discriminatory power, and all three factors were necessary to discriminate all four species groups. The first discriminant factor distinguished *M. kinseyi* and the Appalachian species from the two Rockies species. The second discriminant factor distinguished the two Rockies species from each other, and the third discriminant factor distinguished *M. kinseyi* from the Appalachian species. In three of the four species, unknown individuals grouped with the clouds of specimens belonging to the correct taxa. In one of the Rockies species, the two unknowns (left and right halves of the same specimen) were located between the two Rockies species and their correct taxonomic affinity was not obvious.

Neither the wing-only (Fig. 4, three misidentified individuals) nor the appendage-only (data not shown; 13 misidentified individuals) DFA distinguished the species as readily as the combined analysis (Fig. 3), although unknowns did fall close to their respective taxon clouds. In the wing-only analysis, the first discriminant factor discriminated the Appalachian species and the second factor discriminated one of the Rocky Mountain species, but *M. kinseyi* and the second Rockies species were not distinguished (Fig. 4).

Analyses that included the *S. pinicola* holotype followed the same trend as those that excluded it. Neither the wing-only data (Fig. 4) nor the appendage-only data (in this case also excluding hind leg measurements; figure not shown, 20 misidentified individuals) were as clear in discriminating the four species as the combined analysis (Fig. 5, no misclassed individuals). The two points representing the two halves of the *S. pinicola* holotype were loosely associated with the Appalachian species, but they did not group as closely as did the two unknowns of that same species (Fig. 5).

Discussion

Molecular Data. Despite the growing popularity of DNA barcoding (Hebert et al. 2003), DNA sequence divergence alone is insufficient to recognize species boundaries (Anstead et al. 2002, Johnson et al. 2003, Cognato 2006, Schmidt and Sperling 2008). However, relative distances can be informative (Stern et al. 1997, Favret and Voegtlin 2004b), and there seem to be two distinct classes of genetic similarity within these *Mindarus.* Mitochondrial sequence divergence within distal clades was always $\leq$1%, with the one exception of the *M. kinseyi* clades, which will require further study. In contrast, mitochondrial sequence divergence among the clades labeled as different species was always >3%. This difference is greater than the 2% argued for species recognition by others (Stern et al. 1997, Hebert et al. 2003) and by that criterion suggests there are several undescribed species of *Mindarus* in North America (but see Cognato 2006). The even greater divergence between the Nearctic samples (including 132 collections across the United States and Canada, unpublished data) and the European *M. abietinus* suggests that this latter species is not present in North America (as first hypothesized by Voegtlin [1995]).

Discriminant Functions and Wing Morphometry. The combined data analyses, including both the wing and appendage morphometry, clearly yielded better results than the wing-only analyses. All the univariate appendage data exhibited the same size trend across species, whereas the wing data showed a greater diversity across species: even the Appalachian species, clearly the smallest of the four based on all other measurements, had the longest media (L9). The wing data generally contributed more to the DFA than did the appendage data, with the two highest positive and lowest negative scores of the first three canonical scores all being wing measures, and only six append-

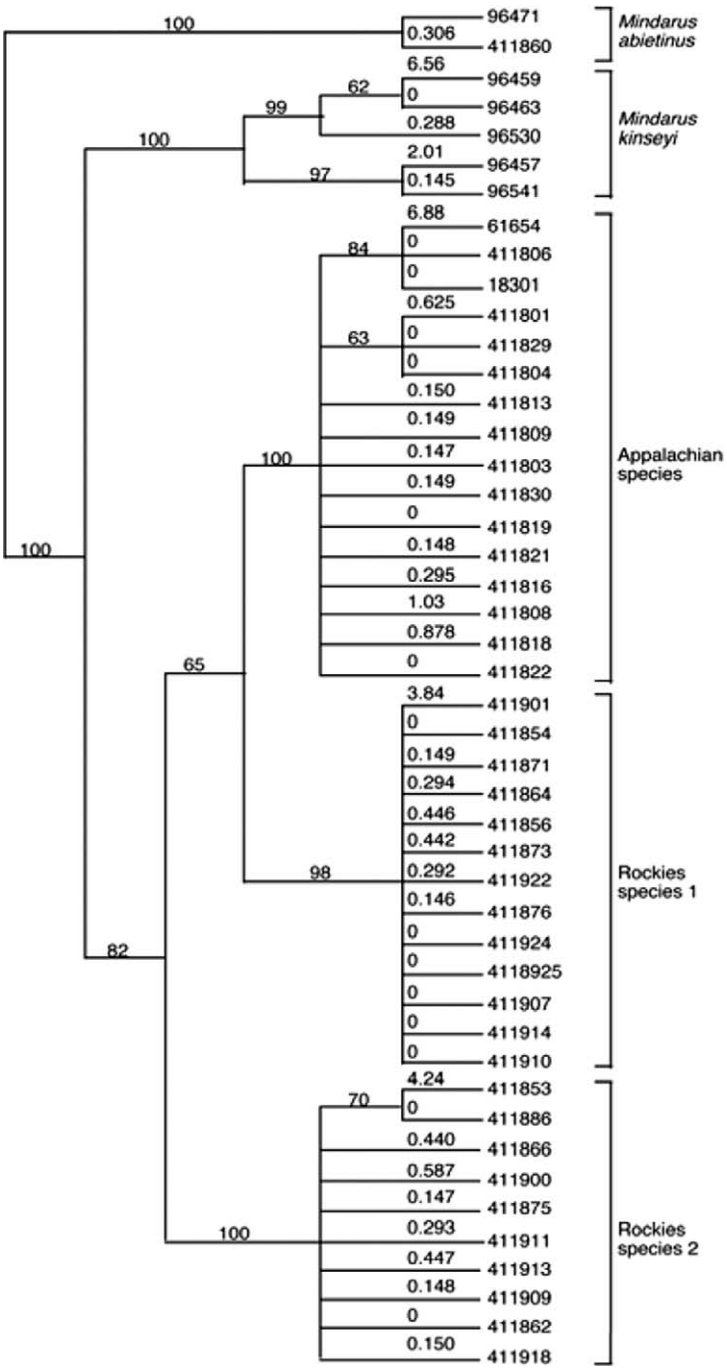

Fig. 2. Unrooted maximum parsimony tree of partial CO1 sequences. *M. abietinus* chosen as outgroup for clarity. Terminals are labeled with INHS specimen catalog numbers. Numbers between terminal branches are percent sequence divergence. Numbers above other branches are bootstrap support values >50.

age measures were part of the 44 canonical scores >1.0 or less than −1.0. Thus, quite a lot of the combined taxonomic signal is present in the wings.

Superficially, it seems the morphometric and molecular data may yield similar relationships between the four species. Specifically, the greatest observed genetic distance is between *M. kinseyi* and the other North American species (Fig. 2). There may be a greater morphometric distance, too, as reflected in the third discriminant score (Fig. 3B). A fuller test of this hypothesis of molecular and morphometric correlation will be made with a larger data set in the future.

Table 3. Canonical scores and measurements for 35 morphometrics in DFA 2

Morphometric	Canonical discriminant function			*S. pinicola* type		Means				ANOVA
	1	2	3	Left side	Right side	Rockies 1 (n = 21)	Appalachian (n = 26)	M. kinseyi (n = 9)	Rockies 2 (n = 18)	
Sensoria	0.768	0.735	0.2	15	13	16.5	11.8	12.9	18.9	***
Antenna 3	−0.852	0.128	0.08	399	391	430	384	396	408	*
Antenna 4	−0.018	1.932	−0.288	192	191	236	190	232	239	***
Antenna 5	0.65	0.103	0.258	199	200	250	212	234	246	***
Antenna 6	−0.229	−1.436	0.228	209	208	254	219	236	245	***
Metafemur	−1.281	0.763	−0.179	na	na	488	464	496	497	
Metatibia	−1.38	0.384	2.311	na	na	811	705	795	817	***
Metatarsus 2	0.957	0.827	−1.51	na	na	229	201	202	238	***
Forefemur	−0.02	0.592	0.509	394	383	487	438	474	507	**
Foretibia	0.358	−3.243	0.665	612	612	697	598	667	688	***
Foretarsus 2	0.241	−0.43	−0.974	179	187	193	169	170	197	***
S1	−1.469	−0.191	0.821	312571	315917	442912	299051	418362	437695	***
S2	−0.426	1.076	0.093	165820	149330	190530	137026	174826	178762	***
S3	0.956	−1.142	1.756	261853	238289	377293	264977	358360	371516	***
A1	0.077	−0.439	0.042	31.1	27.9	29.9	28.1	28.4	29.5	*
A2	0.412	−0.807	0.237	124	125	122	122	122	121	
A3	−2.003	1.509	−0.813	42.8	42.3	42.3	45.1	43.4	44.5	***
A4	−0.726	0.668	0.784	38.8	40.1	29.7	34.3	27.9	30.4	***
A5	1.17	−0.182	0.612	49.7	49.4	48.0	47.4	49.4	48.7	**
L1	−0.397	−6.08	−1.981	795	890	901	751	861	884	***
L2	−1.158	−0.39	−2.368	825	762	900	792	918	907	***
L3	2.656	6.435	2.721	828	817	938	820	941	962	***
L4	0.294	−11.436	−3.033	950	923	1129	959	1093	1120	***
L5	4.831	−1.082	−1.092	1208	1214	1495	1275	1416	1460	***
L6	−0.177	6.454	−0.169	955	971	1098	1003	1078	1158	**
L7	−0.136	0.781	−5.131	692	752	877	689	790	829	***
L8	−3.894	14.978	3.985	1358	1349	1614	1331	1537	1565	***
L9	−0.862	0.782	−0.649	231	356	175	328	171	230	***
L10	−1.603	0.818	0.395	808	748	977	782	949	911	***
L11	2.756	−4.099	−0.514	526	473	504	448	466	468	
L12	−0.445	−1.568	1.468	517	490	694	527	689	664	***
L13	−1.441	−0.749	−0.265	206	221	227	225	254	233	
L14	0.024	−3.706	1.878	670	740	776	702	733	790	*
L15	1.224	−1.884	−0.648	227	252	305	300	296	309	
L16	1.248	−2.065	1.109	253	258	322	295	344	413	***

Sensoria, actual count on flagellomere 3; S1–3, square micrometers; A1–5, degrees; all others, micrometers.
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ in univariate ANOVA.

Aphids have a reduced wing venation and pigmentation (Patch 1909), especially compared with the larger flying insects used in wing-based automated identification, and *Mindarus* venation is reduced even by aphid standards. For successfully diagnosing bumblebee species, Steinhage et al. (2007) used 50 morphometrics and 240 cell areas graded for pigmentation intensity. These 290 characters are far more than the 24 wing characters I used, but it would be difficult to extract many more. Using nonlinear discriminant analysis, as Steinhage et al. (2007) did, may improve the aphid taxonomic resolution. Adding characters from the hind wings may also help, although they are even more reduced than the forewings, containing no closed cells and only one or two forks. On top of reduced venation, aphids may pose greater challenges than other insects with regard to automated identification using wing veins. Babbitt (2008) found that fluctuating asymmetry in the cotton aphid, *Aphis gossypii* Glover, was four times greater than in other insects, suggesting a high level of phenotypic plasticity.

That wing morphometry does contain taxonomic signal is clear, however, and more sophisticated mathematical techniques for character extraction and character analysis may produce better results yet. Given that most of these aphid species were cryptic enough to have gone unnoticed for all this time renders these results all the more compelling.

Resurrection of *M. pinicolus* (Thomas). If *M. abietinus* is not present in North America, then the balsam twig aphid requires a different scientific name. Most of the research on the balsam twig aphid has involved what I here have referred to as the Appalachian species (e.g., Nettleton and Hain 1982, Kleintjes et al. 1999, Fondren and McCullough 2003, Berthiaume et al. 2007). Meanwhile, the Nearctic *S. pinicola* can no longer be considered a synonym of the absent European species. Given the proximity of the type locality of *S. pinicola* (Illinois) to the Appalachians relative to the other American regions of *Mindarus* endemism (i.e., the Rocky Mountains and westward), it seems most likely that *S. pinicola* is this Appalachian species. The discriminant function analyses presented here solidify it by grouping both halves of the holotype specimen of *S. pinicola* closest to the Appalachian species. I here resurrect *S. pinicola* Thomas under the name *M. pinicolus* (Thomas).

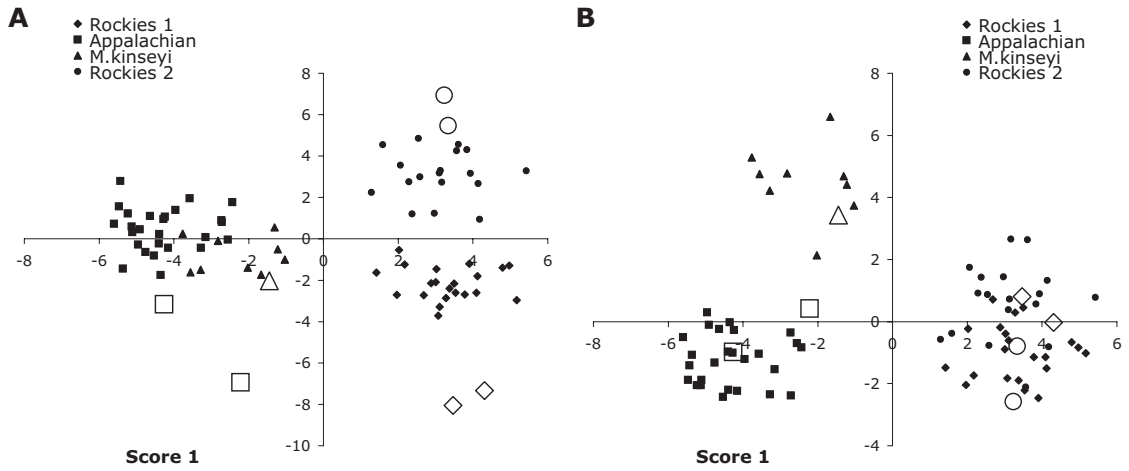

Fig. 3. DFA of all specimens except the holotype using all wing and appendage data. Three dimensions of discriminant function data represented on two charts. Each point represents the left or right hand of a specific individual. Solid icons represent specimens analyzed as groups. Open icons represent specimens added to the analysis individually, the icon shape corresponding to the appropriate species. (A) Discriminant scores 1 and 2. (B) Scores 1 and 3.

All measurements below are in micrometers.

Mindarus pinicolus (Thomas), reinstated

Schizoneura pinicola Thomas 1879: 137.

Mindarus abietinus Koch. Patch 1910: 242 erroneously proposed synonymy.

Apterous Vivipara. Measurements count $n = 32$. Body length 1180–2570 (mean = 2020) (Fig. 6C). *Head:* flagellomere III 134–336 (mean = 205); IV 60–194 (mean = 107); V 100–194 (mean = 129); VI 131–220 (mean = 162); VI base 106–180 (mean = 133); secondary antennal rhinaria lacking (a single specimen with 2); six setae on tip of processus terminalis; compound eyes absent, reduced, or present, triommatidia always present; rostrum extending to metacoxae; two accessory setae on ultimate rostral segment (URS) (rarely 0 or 1); URS 57.4–90.1 (mean = 75.9). *Thorax:* profemur 213–391 (mean = 288); protibia 254–473 (mean = 341); protarsus II 105–176 (mean = 134); metafemur 248–471 (mean = 343); metatibia 352–655 (mean = 462); metatarsus II 134–214 (mean = 164); tarsus I triangular with four terminal setae and sensory peg. *Abdomen:* wax gland plates (Fig. 6E) typical in morphology, variable in size, with single seta on margin, on abdominal segment I 0–6, II 0–6, III 0–6, IV 0–6, V 2–6, VI 6, VII 4, VIII 2; dorsal abdominal setae occasionally located on scleroites; genital plate with two setae on anterior margin and 8–15 setae variably placed but mostly aligned along posterior margin.

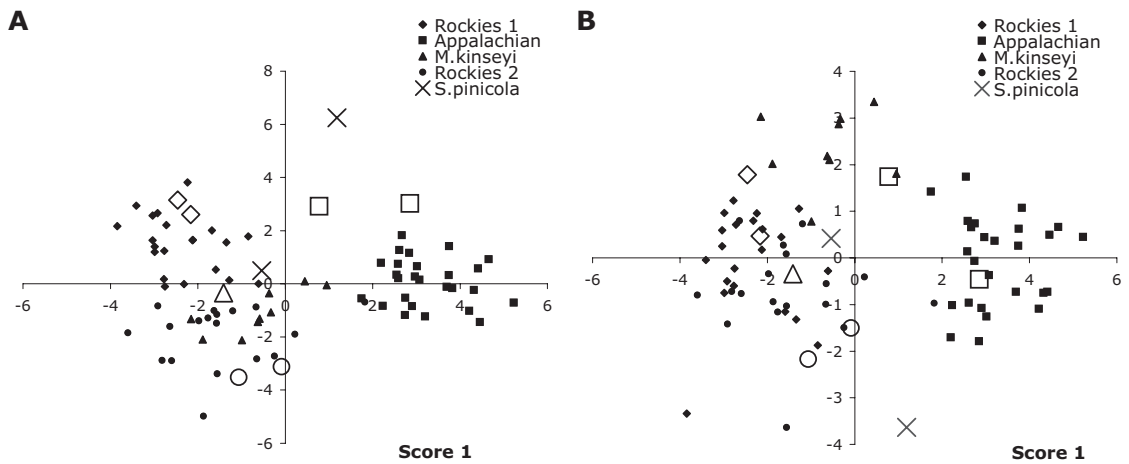

Fig. 4. DFA of all specimens using wing data only. Three dimensions of discriminant function data represented on two charts. Each point represents the left or right hand of a specific individual. Solid icons represent specimens analyzed as groups. Open icons represent specimens added to the analysis individually, the icon shape corresponding to the appropriate species. The left and right sides of the *S. pinicola* holotype were each added individually. (A) Discriminant scores 1 and 2. (B) Scores 1 and 3.

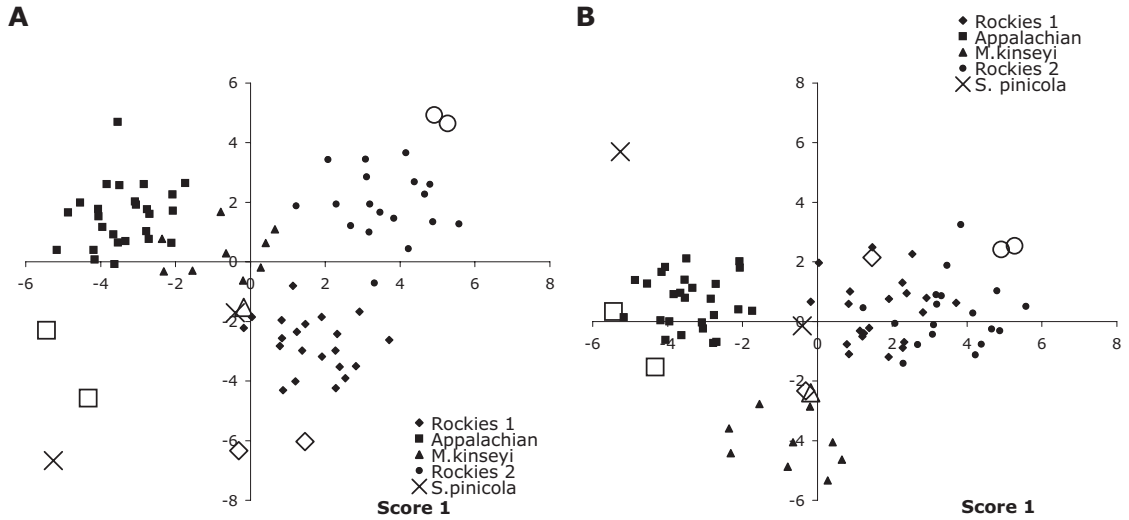

Fig. 5. DFA of all specimens using wing and appendage data but omitting hind leg data. Three dimensions of discriminant function data represented on two charts. Each point represents the left or right hand of a specific individual. Solid icons represent specimens analyzed as groups. Open icons represent specimens added to the analysis individually, the icon shape corresponding to the appropriate species. The left and right sides of the *S. pinicola* holotype were each added individually. (A) Discriminant scores 1 and 2. (B) Scores 1 and 3.

Alate Vivipara. Measurements count $n = 41$. Body length 1740–2770 (mean = 2170) (Fig. 6B). *Head:* flagellomere III 414–636 (mean = 383); IV 144–237 (mean = 193); V 178–251 (mean = 212); VI 190–255 (mean = 220); VI base 153–230 (mean = 189); 9–18 (mean = 12) rhinaria on flagellomere III; longest seta on flagellomere III 10.6–18.7 (mean = 13.7); six setae on tip of processus terminalis; compound eyes and triommatidia present; rostrum extending to metacoxae; two accessory setae on URS (one specimen with 3); URS 74.6–92.0 (mean = 83.9). *Thorax:* profemur 372–544 (mean = 444); protibia 520–712 (mean = 607); protarsus II 154–192 (mean = 171); metafemur 367–753 (mean = 471); metatibia 611–849 (mean = 716); metatarsus II 176–223 (mean = 203); seta on mid-dorsal aspect of metatibia 12.5–23.6 (mean = 18.8); mesothoracic wing 2190–3490 (mean = 2860). *Abdomen:* seta on tergite V 10.3–19.6 (mean = 14.4); sclerites cover large portion of each abdominal tergum, variable in width, much shorter length on terga I and II, with setae located within wax glands on posterior margin of sclerites (Fig. 6A); genital plate with two setae on anterior margin and 8–15 setae variably placed but mostly aligned along posterior margin; anal plate with two unsclerotized tubercles; cauda small and knobbed, with or without slight constriction basal to knob.

Apterous Ovipara. Measurements count $n = 10$. Body length 770-1040 (mean = 950) (Fig. 6G). *Head:* flagellomere III 42.5–49.6 (mean = 45.5); IV 19.0–33.3 (mean = 26.7); V 44.8–58.8 (mean = 52.6); VI 87.0–99.0 (mean = 92.2); secondary antennal rhinaria lacking; compound eyes absent, triommatidia present; two accessory setae on URS (sometimes 1); URS 44.8–52.0 (mean = 47.7). *Thorax:* profemur 109–134 (mean = 125); protibia 128–148 (mean = 135); protarsus II 56.8–77.0 (mean = 67.8); metafemur 133–158 (mean = 147); metatibia 168–202 (mean = 181); metatarsus II 72.8–82.8 (mean = 78.5). *Abdomen:* two large wax glands on ventral abdominal V, each with a single faceted tubercle centrally located (Fig. 6D). Other wax gland plates absent.

Apterous Male. Measurements count $n = 9$. Body length 609–724 (mean = 671) (Fig. 6H). *Head:* flagellomere III 55.8–73.1 (mean = 66.9); IV 30.9–43.3 (mean = 37.5); V 48.4–59.7 (mean = 55.6); VI 85.9–99.2 (mean = 92.7); secondary antennal rhinaria lacking on flagellomere III, but present on flagellomere IV (1–2), V (2–4), and VI base (3–5) (Fig. 6F); flagellomere III smooth, IV-VI with spicules; compound eyes absent, triommatidia present; 0, 1, or two accessory setae on URS; URS 36.2–46.9 (mean = 40.6). *Thorax:* profemur 118–129 (mean = 124); protibia 128–148 (mean = 134); protarsus II 53.9–70.4 (mean = 61.3); metafemur 136–151 (mean = 144); metatibia 169–182 (mean = 176); metatarsus II 67.1–81.4 (mean = 72.5). *Abdomen:* all dorsal abdominal segments sclerotized.

Discussion

With the exception of *M. kinseyi*, *Mindarus* species are thought to have a reduced life cycle with a single apterous fundatrix generation giving rise to the alate vivipara. Voegtlin (1995) based his description of *M. kinseyi* partly on its having supernumerary apterous generations. He found that as a general rule, fundatrices lacked compound eyes and nonfundatrix apterous viviparae had them. *M. pinicolus* apterae also fall within this range of morphological diversity. Those lacking compound eyes are more likely to have shorter appendages and a full complement of six wax gland plates on abdominal seg-

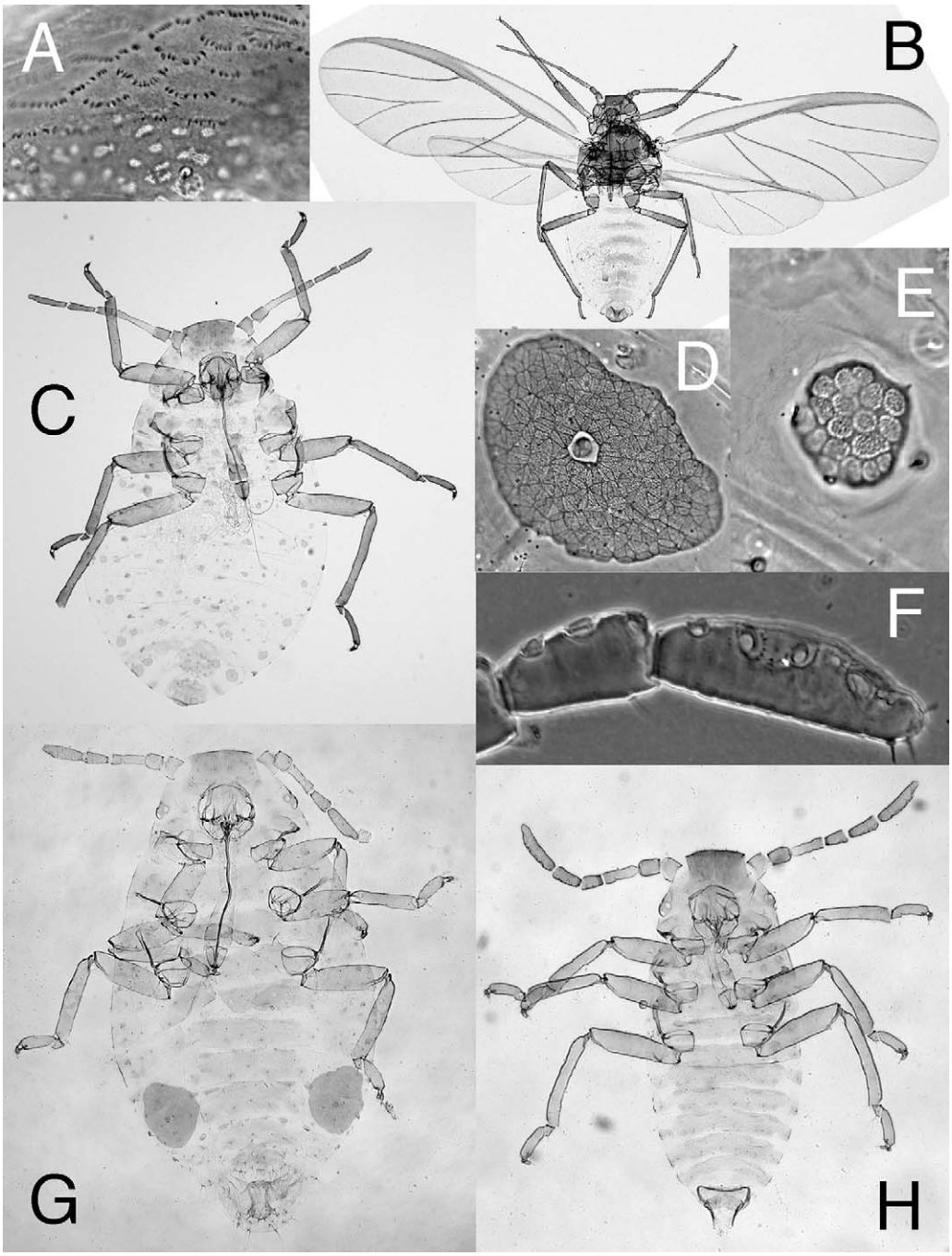

Fig. 6. *Mindarus pinicolus.* (A) Detail of dorsal sclerite and wax gland of alate vivipara specimen USNM398858. (B) Alate vivipara specimen USNM398820. (C) Apterous vivipara specimen INHS96549. (D) Detail of ventral abdominal wax gland of ovipara specimen INHS48371. (E) Detail of dorsal abdominal wax gland plate of apterous vivipara specimen INHS96549. (F) Detail of terminal antennal segments of male specimen INHS48357. (G) Ovipara specimen INHS48368. (H) Male specimen INHS48364.

ments I-VI, although some of these glands may consist of as few as a single cell. Those with compound eyes are more likely to have fewer wax gland plates on the anterior abdominal segments and longer appendages. There are intermediate forms, however, including apterae with compound eyes reduced to 1–4 ommatidia and individuals with the reversed wax gland plate–compound eye tendencies.

Most historical published references to *M. abietinus* from *Abies* in eastern North America should be re-

ferred to *M. pinicolus.* There is much knowledge on the biology of the species within these many publications. The species, the balsam twig aphid, represents a case where the insect's common name is more stable than its scientific name.

Unfortunately, Thomas (1879) collected the lone (type) specimen of *M. pinicolus* on a white pine (*Pinus strobus* L.), probably an incidental host. It is unfortunate that an aphid species should be named for a plant it does not colonize regularly. Neither *Abies* nor *Picea* occur natively in Carbondale, IL, so Thomas's specimen probably came from a cultivated or transplanted ornamental host. *Mindarus* colonies are known to persist many years, even decades, on individual trees in non-native areas. For example, the colony from which specimen 179,776 (Rockies 2) was taken has persisted on the same tree in Champaign, IL, for >20 yr.

Diagnosis. *M. pinicolus* apterae always have more wax gland plates on terminal abdominal segments than do specimens of European *M. abietinus* (fig. 7 in Voegtlin 1995). *M. pinicolus* is the only *Mindarus* species on native eastern North American *Abies* (*Abies balsamea* (L.) Miller and *Abies fraseri* (Pursh) Poiret). I have seen other *Mindarus* species on *Abies concolor* (Gordon et Glendinning) Lindley ex Hildebrand (a western North American native sometimes grown ornamentally in the eastern United States) and *Picea* from eastern North America. Descriptions of these species and a key will be published separately.

Material Measured. All material is deposited in the insect collection of the Illinois Natural History Survey (INHS, Champaign, IL) and the U.S. National Museum of Natural History Aphid Collection (USNM, Beltsville, MD). Numbers refer to data-based catalog numbers unique to these collections. HOLOTYPE: alate vivipara, INHS199996, USA, IL, Carbondale, coll. 20 April 1878 by C. Thomas on *Pinus strobus.* Apterous viviparae: INHS49002–49003, USA, MI, Kalamazoo Co., W. K. Kellog Forest, coll. 50 May 1995 by D. J. Voegtlin on *A. balsamea;* INHS49004, coll. 27 May 1995; INHS49005, coll. 31 May 1995. INNHS96550–96551, USA Michigan, Kalamazoo Co., 42.36°x-85.35°, coll. 26 May 2005 by D. J. Voegtlin on *A. balsamea.* INHS96546, 96549, USA, MI, Roscommon Co., 44.36°x-84.61°, coll. 26 May 2005 by D. J. Voegtlin on *A. balsamea.* INHS411811, USA, NY, Essex Co., 44.23°x-73.95°, coll. 18 June 2006 by C. Favret and S. Favret on *A. balsamea.* USNM398826, USA, NY, Essex Co., 44.32°x-73.95°, coll 18 June 2006 C. Favret and S. Favret on *A. balsamea.* USNM396704–396705, USA, NY, Farmingdale, coll. 20 May 1954 by G. V. Johnson on *Abies.* USNM396706–396707, USA New York, Tompkins Co., Enfield, coll. 6 June 1967 by W. T. Johnson on *A. balsamea.* INHS411815, USA, NC, Haywood Co., 35.69°x-83.13°, coll. 11 June 2006 by C. Favret on *A. fraseri.* INHS96494, USA, VT, Lamoille Co., 44.44°x-72.68°, coll. 22 May 2005 by R. S. Kelley on *A. balsamea.* USNM398715–398723, 398890–398891, USA, VT, Lamoille Co., Stowe, 44.44°x-72.68°, coll. 22 May 2005 by R. S. Kelley on *A. balsamea.* INHS411827, USA, VA, Grayson Co., 36.65°x-81.58°, coll. 15 June 2006 by C. Favret and S. Favret on *A. fraseri.* INHS96548, USA, WI, Lincoln Co., 45.54°x-89.68°, coll. 25 May 2005 by D. J. Voegtlin on *A. balsamea.* INHS96547, USA, WI, Price Co., 45.55°x-90.13°, coll. 25 May 2005 by D. J. Voegtlin on *A. balsamea.* Alate viviparae: INHS411671, INHS411819, Canada, Quebec, 47.59°x-68.72°, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411801, Canada, Quebec, 47.78°x-70.23°, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411813, Canada, Quebec, 47.51°x-70.51°, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411683, INHS411829, USA, ME, Aroostook Co., 47.29°x-68.50°, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411805, INHS411824, USA, ME, Aroostook Co., 46.93°x-68.53°, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411808, USA, ME, Aroostook Co., 45.66°x-68.28°, coll. 20 June 2006 by C. Favret and S. Favret on *A. balsamea,* INHS411809, USA, ME, Aroostook Co., 46.66°x-68.24°, coll. 20 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411818, USA, ME, Aroostook Co., 46.24°x-68.34°, coll. 20 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411822, USA, NH, Grafton Co., 44.10°x-71.84°, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411821, USA, NY, Oneida Co., 43.59°x-75.12°, coll. 17 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411804, USA, NY, St. Lawrence Co., 44.13°x-74.63°, coll. 18 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS18301, INHS20037–20040, USA, NC, Haywood Co., 35.58°x-83.07°, coll. 31 May 2003 by C. Favret on *A. fraseri.* INHS411806, USA, NC, Haywood Co., 35.58°x-83.07°, coll. 14 June 2006 by C. Favret and S. Favret on *A. fraseri.* INHS61606, INHS61608, INHS61654, coll. 18 May 2004. INHS61859, coll. 31 May 2003. INHS33618, USA, NC, Swain Co., 35.60°x-83.46°, coll. 4 June 2003 by C. Favret on *A. fraseri.* INHS33636, USA, NC, Swain Co., 35.54°x-83.49°, coll. 16 July 2003 by C. Favret on *A. fraseri.* INHS411803, USA, VT, WA Co., 44.08°x-72.86°, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411803, USA, VT, WA Co., 44.08°x-72.86°, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411816, USA, VT, Addison Co., 44.00°x-73.02°, coll. 18 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411830, USA, VT, Addison Co., 43.94°x-72.95°, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea.* INHS411807, USA, VA, Grayson Co., 36.65°x-81.58°, coll. 15 June 2006, by C. Favret and S. Favret on *A. fraseri.* Oviparae: [note: although the following examined oviparae and males were collected on *Abies grandis,* a western North American species, concurrently collected alatae are clearly *M. pinicolus* and the absence of wax gland plates indicates that the oviparae cannot be either of the other *A. grandis* feeding species, *M. victoria* Essig or *M. kinseyi*] USNM396708, USA, MD, Oakland, coll. 5 June 1969 by F. D. Custer and F. Langford. INHS48363–48371, USA, MI, Kalamazoo Co., W. K. Kellog Forest, coll. 11 June 1993 by D. J. Voegtlin on *A. grandis.* Males: INHS48357–48361, USA, MI, Kalamazoo Co., W. K. Kellog Forest, coll. 6 May 1993 by R. Lawrence on *A. grandis.* INHS48363, INHS48365–48366, INHS48368,

INHS48370, USA, MI, Kalamazoo Co., W. K. Kellog Forest, coll. 11 June 1993 by D. J. Voegtlin on *A. grandis.*

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References Cited

Anstead, J. A., J. D. Burd, and K. A. Shufran. 2002. Mitochondrial DNA sequence divergence among *Schizaphis graminum* (Hemiptera: Aphididae) clones from cultivated and non-cultivated hosts: haplotype and host associations. Bull. Entomol. Res. 92: 17–24.

Babbitt, G. A. 2008. How accurate is the phenotype? – An analysis of developmental noise in a cotton aphid clone. BMC Dev. Biol. 8: 19.

Bhanu, B., R. Li, J. Heraty, and E. Murray. 2008. Automated classification of skippers based on parts representation. Am. Entomol. 54: 228–231.

Baylac, M., C. Villemant, and G. Simbolotti. 2003. Combining geometric morphometrics with pattern recognition for the investigation of species complexes. Biol. J. Linn. Soc. 80: 89–98.

Berthiaume, R., C. Hébert, and C. Cloutier. 2007. Comparative use of *Mindarus abietinus* (Homoptera: Aphididae) by two coccinellids (Coleoptera: Coccinellidae), the native *Anatis mali* and the exotic *Harmonia axyridis,* in a Christmas tree plantation. Environ. Entomol. 36: 319–328.

Blackman, R. L. 1987. Morphological discrimination of a tobacco-feeding form of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and a key to New World *Myzus* (*Nectarosiphon*) species. Bull. Entomol. Res. 77: 713–730.

Brown, P. A., and R. L. Blackman. 1994. Morphometric variation in the *Geoica utricularia* (Homoptera: Aphididae) species group on *Pistacia* (Anacardaceae), with descriptions of new species and a key to emigrant alatae. Syst. Entomol. 19: 119–132.

Clement, M., D. Posada, and K. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657–1660.

Cognato, A. 2006. Standard percent DNA sequence difference for insects does not predict species boundaries. J. Econ. Entomol. 99: 1037–1045.

Corpuz-Raros, L. A., and E. F. Cook. 1974. A revision of North American *Capitophorus* van der Goot and *Pleotrichophorus* Börner (Homoptera: Aphididae). Smithson. Contrib. Zool. 156: 1–143.

Damsteegt, V. D., and D. J. Voegtlin. 1990. Morphological and biological variation among populations of *Aulacorthum solani* (Homoptera: Aphididae): the vector of soybean dwarf virus. Ann. Entomol. Soc. Am. 83: 949–955.

Eastop, V. F. 1971. Keys for the identification of *Acyrthosiphon* (Hemiptera: Aphididae). Bull. Br. Mus. Nat. Hist. (Entomol.) 26: 1–115.

Ehler, L. E., and M. G. Kinsey. 1995. Ecology and management of *Mindarus kinseyi* Voegtlin (Aphidoidea: Mindaridae) on white-fir seedlings at a California forest nursery. Hilgardia 62: 1–62.

Favret, C. 2005. A new non-destructive DNA extraction and specimen clearing technique for aphids (Hemiptera). Proc. Entomol. Soc. Wash. 107: 469–470.

Favret, C., and C. Nielsen. 2008. A new species of *Mindarus* (Hemiptera: Aphididae) on the endangered Guatemalan fir. Ann. Entomol. Soc. Am. 101: 833–836.

Favret, C., and D. J. Voegtlin. 2004a. A revision of the *Cinara* species (Hemiptera: Aphididae) of the United States pinyon pines. Ann. Entomol. Soc. Am. 97: 1165–1197.

Favret, C., and D. J. Voegtlin. 2004b. Speciation by host-switching in pinyon *Cinara* (Insecta: Hemiptera: Aphididae). Mol. Phylogenet. Evol. 32: 139–151.

Favret, C., J. F. Tooker, and L. M. Hanks. 2004 Iowana frisoni Hottes (Hemiptera: Aphididae) redescribed with notes on its biology. Proc. Entomol. Soc. Wash. 106: 26–34.

Fondren, K. M., and D. C. McCullough. 2003. Phenology and density of balsam twig aphid, *Mindarus abietinus* Koch (Homoptera: Aphididae) in relation to bud break, shoot damage, and value of fir Christmas trees. J. Econ. Entomol. 98: 1760–1769.

Hand, S. C. 1986. The use of multivariate morphometric methods in the separation of alate morphs of the rose-grain aphid, *Metapolophium dirhodum.* Ann. Appl. Biol. 109: 19–31.

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.

Johnson, K.P.R., R. J. Adams, R.D.M. Page, and D. H. Clayton. 2003. When do parasites fail to speciate in response to host speciation? Syst. Biol. 52: 37–47.

Kleintjes, P. K., E. E. Lemoine, J. Schroeder, and M. J. Solensky. 1999. Comparison of methods for monitoring *Mindarus abietinus* (Homoptera: Aphididae) and their potential damage in Christmas tree plantations. J. Econ. Entomol. 92: 638–643.

Koch, C. L. 1857. Die Pflanzenläuse Aphiden, getreu nach dem Leben abgebildet und beschreiben, volume 9, pp. 275–336. J. L. Lotzbeck, Nürnberg.

Lozier, J. D., R. G. Foottit, G. L. Miller, N. J. Mills, and G. K. Roderick. 2008. Molecular and morphological evaluation of the aphid genus *Hyalopterus* Koch (Insecta: Hemiptera: Aphididae), with a description of a new species. Zootaxa 1688: 1–19.

Nettleton, W. A., and F. P. Hain. 1982. The life history, foliage damage and control of the balsam twig aphid, *Mindarus abietinus* Koch (Homoptera: Aphididae) in Fraser fir Christmas tree plantations of western North Carolina. Can. Entomol. 114: 155–162.

Patch, E. M. 1909. Homologies of the wing veins of the Aphididae, Psyllidae, Aleurodidae, and Coccidae. Ann. Entomol. Soc. Am. 2: 101–129.

Patch, E. M. 1910. Four rare aphid genera from Maine. Bull. Maine Agric. Exp. Stn. 182: 241–248.

Pimentel, R. A. 1992. An introduction to ordination, principal components analysis and discriminant analysis, pp. 11–28. *In* J. T. Sorenson and R. G. Foottit [eds.], Ordination in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationals. Elsevier, Amsterdam, The Netherlands.

Robinson, A. G. 1985. Annotated list of *Uroleucon* (*Uroleucon*, *Uromelan*, *Satula*) (Homoptera: Aphididae) of America north of Mexico, with keys and descriptions of new species. Can. Entomol. 117: 1029–1054.

Schmidt, B. C., and F.A.H. Sperling. 2008. Widespread decoupling of mtDNA variation and species integrity in *Grammia* tiger moths (Lepidoptera: Noctuidae). Syst. Entomol. 33: 613–634.

Sidebottom, J. R. 2008. Crop profile for Christmas trees in North Carolina (Mountains). U.S. Dep. Agric. Southern Region IPM Center Crop Profile. (http://www.ipmcenters.org/cropprofiles/docs/NCchristmastrees.pdf).

Sokal, R. R. 1952. Variation in a local population of *Pemphigus*. Evolution 6: 296–315.

Sokal, R. R. 1962. Variation and covariation of characters of alate *Pemphigus populi-transversus* in eastern North America. Evolution 16: 227–245.

Sorensen, J. T. 1994. A revision of the aphid genus *Essigella* (Homoptera: Aphididae: Lachninae): its ecological associations with, and evolution on, Pinaceae hosts. Pan-Pac. Entomol. 70: 1–102.

Steinhage, V., S. Schröder, K.-H. Lampe, and A. B. Cremers. 2007. Automated extraction and analysis of morphological features for species identification, pp. 115–129. *In* N. MacLeod [ed.], Automated taxon identification in systematics: theory, approaches and applications. CRC, Boca Raton, FL.

Stern, D. L., S. Aoki, and U. Kurosu. 1997. Determining aphid taxonomic affinities and life cycles with molecular data: a case study of the tribe Cerataphidini (Hormaphididae: Aphidoidea: Hemiptera). Syst. Entomol. 22: 81–96.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, MA.

Thomas, C. 1879. Noxious and beneficial insects of the state of Illinois. Report of the State Entomologist (Illinois) 8: 1–212.

Tofilski, A. 2007. Automatic measurement of honeybee wings, pp. 289–298. *In* N. MacLeod [ed.], Automated taxon identification in systematics: theory, approaches and applications. CRC, Boca Raton, FL.

Voegtlin, D. J. 1995. Notes on *Mindarus* spp. (Homoptera: Aphididae) of North America with descriptions of two new species. Proc. Entomol. Soc. Wash. 97: 178–196.

Watson, G. W., D. J. Voegtlin, S. T. Murphy, and R. G. Foottit. 1999. Biogeography of the *Cinara cupressi* complex (Hemiptera: Aphididae) on Cupressaceae, with description of a pest species introduced into Africa. Bull. Entomol. Res. 89: 271–283.

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