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

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Ann. Entomol. Soc. Am. 102(6): 970–981 (2009)

**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. *Schizoneurapinicola* 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 between each individual and the various centroids.
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).

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

| INHS   | Species | Country       | State or province | County     | Latitude | Longitude | Collector(s) | Date        | Host       | GenBank |
|--------|---------|---------------|-------------------|------------|----------|-----------|--------------|-------------|------------|---------|
| 411,391| M. abietinus | U.S.A. North Carolina | Haywood       | 35.58     | -83.07   | C. Favret | 05/21/2003 | Abies fraser | FJ68253    |
| 411,801| M. abietinus | U.S.A. Missouri | Arkansas       | 36.47     | -90.55   | C. Favret | 05/18/2004 | A. fraser   | FJ68251    |
| 411,803| M. abietinus | U.S.A. Missouri | Arkansas       | 36.27     | -90.36   | C. Favret | 05/18/2004 | A. fraser   | FJ68251    |
| 411,804| M. abietinus | U.S.A. Missouri | Arkansas       | 36.47     | -90.55   | C. Favret | 05/18/2004 | A. fraser   | FJ68251    |
| 411,805| M. abietinus | U.S.A. Missouri | Arkansas       | 36.47     | -90.55   | C. Favret | 05/18/2004 | A. fraser   | FJ68251    |

### 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 Voeglin (2004b). Forward and reverse sequences were combined, and edited sequences aligned, using Sequencher 4.7 soft-

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

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**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).
November 2009

FAVRET: Mindarus Wing Morphometry

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

| No. | Included data | Type included? | Misclassified individuals | Related figure |
|-----|---------------|----------------|---------------------------|----------------|
| 1   | Yes           | No             | Yes                       | 3              |
| 2   | Yes           | Yes            | No                        | 0              |
| 3   | No            | Yes, no hind leg | Yes                      | 15             |
| 4   | Yes           | Yes, no hind leg | Yes                      | 6              |
| 5   | No            | Yes, no hind leg | Yes                      | 20             |

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

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 clad 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 morphology had the clearest discrimination (Fig. 3) with no individual being misclassified 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 misclassified 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 2004), and there seem to be two distinct classes of genetic similarity within these Mindarus. Mitochondrial sequence divergence within distal clades was always ≈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-
age measures were part of the 44 canonical scores $\geq 1.0$ 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.

Fig. 2. Unrooted maximum parsimony tree of partial COI 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 $\geq 50$. 

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**Reference:**

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Aphids have a reduced wing venation and pigmentation (Patch 1909), especially compared with the larger flying insects used in wing-based automated identification, and Mandaruss 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.

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) | Appalachia 2 (n = 20) | M. kinsleyi (n = 9) | Rockies 2 (n = 18) |
| Sensoria    | 0.788 | 0.735 | 0.2 | 15 | 13 | 16.5 | 11.5 | 12.9 | 19.8 | *** |
| Antennae 3  | -0.852 | 0.128 | -0.08 | 390 | 301 | 430 | 384 | 396 | 406 | ** |
| Antennae 4  | -0.018 | 1.932 | -0.258 | 192 | 136 | 236 | 190 | 230 | 23 | *** |
| Antennae 5  | 0.65 | 0.103 | 0.258 | 199 | 200 | 250 | 212 | 234 | 246 | *** |
| Antennae 6  | -0.229 | -1.436 | -0.228 | 209 | 208 | 254 | 219 | 236 | 248 | *** |
| Metalfemur  | -1.251 | 0.763 | -0.179 | na | na | 488 | 464 | 496 | 497 | |
| Metaltibia  | -1.38 | 0.394 | 2.311 | na | na | 811 | 705 | 795 | 817 | *** |
| Metatarsus 2 | 0.957 | 0.827 | -1.53 | na | na | 229 | 201 | 202 | 238 | *** |
| Forefemur   | -0.02 | 0.592 | 0.509 | 394 | 383 | 487 | 438 | 474 | 507 | *** |
| Forethalia  | 0.385 | -3.243 | 0.665 | 612 | 612 | 697 | 598 | 667 | 688 | *** |
| Foretarsus 2 | 0.241 | -0.43 | -0.974 | 179 | 187 | 193 | 189 | 170 | 197 | *** |
| S1          | -1.469 | -0.191 | 0.821 | 312571 | 315917 | 442912 | 390041 | 418362 | 437695 | *** |
| S2          | -0.426 | 1.076 | 0.093 | 165920 | 149393 | 190050 | 137026 | 174826 | 175762 | *** |
| S3          | 0.956 | -1.142 | 1.756 | 261853 | 238289 | 377293 | 326477 | 335360 | 371516 | *** |
| A1          | 0.077 | -0.430 | 0.042 | 31.1 | 27.9 | 29.9 | 28.1 | 25.4 | 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 | 0.734 | 38.5 | 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          | -1.138 | -0.39 | -2.308 | 825 | 752 | 900 | 792 | 918 | 907 | *** |
| L3          | 2.656 | 6.435 | 7.212 | 828 | 817 | 938 | 820 | 941 | 962 | *** |
| L4          | 0.294 | -11.136 | 0.033 | 950 | 923 | 1129 | 959 | 1093 | 1120 | *** |
| L5          | 4.531 | -1.082 | -1.902 | 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 | 1338 | 1349 | 1614 | 1331 | 1537 | 1565 | *** |
| L9          | -0.962 | 0.782 | -0.649 | 311 | 326 | 175 | 149 | 171 | 230 | *** |
| L10         | -1.603 | 0.815 | 0.395 | 808 | 738 | 977 | 782 | 949 | 911 | *** |
| L11         | 2.756 | -4.099 | -0.514 | 526 | 473 | 504 | 448 | 466 | 468 | *** |
| L12         | -0.445 | -1.586 | 1.468 | 517 | 490 | 694 | 527 | 659 | 664 | *** |
| L13         | -1.441 | -0.749 | -0.205 | 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 < 0.1, and ***P < 0.001 in univariate ANOVA.

Resurrection of *M. pinicola* (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 Mandaruss 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. pinicola* (Thomas).
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 1150–2570 (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 106–194 (mean = 129); secondary antennal rhinaria lacking (a single specimen with 2); six setae on tip of processus terminalis; compound eyes absent, reduced, or present, triomatridia 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 sclerites; genital plate with two setae on anterior margin and 8–15 setae variably placed but mostly aligned along posterior margin.

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**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.

**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.
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 metafemur 367–753 (mean = 471); metafemur 118–129 (mean = 116.3); metafemur 136–158 (mean = 147); metatarsus II 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 $n = 41$. 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); metatibia 133–158 (mean = 147); 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 55.9–99.2 (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); metatarsus II 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. Vogeitlin (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. pinicola* 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-
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 (Rocks 2) was taken has persisted on the same tree in Champaign, IL, for >20 yr.

**Diagnosis.** *M. pinicolus* apterous always have more wax gland plates on terminal abdominal segments than do specimens of *M. abietinus* (fig. 7 in Voegtlin 1995). *M. pinicolus* is the only *Mindarus* species on native eastern North American *Abies* (*A. balsamea* (L.) Miller and *A. fraseri* (Pursh) Poir.) 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 1875 by C. Thomas on *Pinus strobus*. Apterous viviparae: INHS49002–49003, USA, MI, Kalamazoo Co., W. K. Kellogg Forest, coll. 50 May 1995 by D. J. Voegtlin on *A. balsamea*. INHS49004, coll. 27 May 1995; INHS49005, coll. 31 May 1995; INHS65560–96551, USA Michigan, Kalamazoo Co., 42.36ºx-85.35º, coll. 26 May 2005 by D. J. Voegtlin on *A. balsamea*. INHS65646, 96549, USA, MI, Roscommon Co., 44.36ºx-84.61º, coll. 26 May 2005 by D. J. Voegtlin on *A. balsamea*. INHS411811, USA, NY, Essex Co., 44.23ºx-73.95º, coll. 18 June 2006 by C. Favret and S. Favret on *A. balsamea*. USNM398826, USA, NY, Essex Co., 44.32ºx-73.95º, coll. 18 June 2006 C. Favret and S. Favret on A. balsamea. USNM39704–397605, USA, NY, Farmingdale, coll. 20 May 1954 by G. V. Johnson on *Abies*. USNM396706–397077, USA New York, Tompkins Co., Enfield, coll. 6 June 1967 by W. T. Johnson on *A. balsamea*. INHS411815, USA, NC, Haywood Co., 35.69ºx-83.13º, coll. 11 June 2006 by C. Favret on *A. fraseri*. INHS96494, USA, VT, Lamoille Co., 44.44ºx-72.68º, coll. 22 May 2005 by R. S. Kelley on *A. balsamea*. USNM398715–398723, 398990–398991, USA, VT, Lamoille Co., Stowe, 44.44ºx-72.68º, coll. 22 May 2005 by R. S. Kelley on *A. balsamea*. INHS411827, USA, VA, Grayson Co., 36.65ºx-81.58º, coll. 15 June 2006 by C. Favret and S. Favret on *A. fraseri*. INHS96548, USA, WI, Lincoln Co., 45.54ºx-89.68º, coll. 25 May 2005 by D. J. Voegtlin on *A. balsamea*. INHS96547, USA, WI, Price Co., 45.55ºx-90.13º, coll. 25 May 2005 by D. J. Voegtlin on *A. balsamea*. Alate viviparae: INHS411671, INHS411819, Canada, Quebec, 47.59ºx-67.72º, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411801, Canada, Quebec, 47.78ºx-70.23º, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411813, Canada, Quebec, 47.51ºx-70.51º, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411683, INHS411829, USA, ME, Aroostook Co., 47.29ºx-68.50º, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411805, INHS411824, USA, ME, Aroostook Co., 46.93ºx-68.53º, coll. 21 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411808, USA, ME, Aroostook Co., 45.66ºx-68.28º, coll. 20 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411809, USA, ME, Aroostook Co., 46.66ºx-68.24º, coll. 20 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411818, USA, ME, Aroostook Co., 46.24ºx-68.34º, coll. 20 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411822, USA, NH, Grafton Co., 44.10ºx-71.84º, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411821, USA, NY, Oneida Co., 43.59ºx-75.12º, coll. 17 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411804, USA, NY, St. Lawrence Co., 44.13ºx-74.63º, coll. 18 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS18301, INHS20037–20040, USA, NC, Haywood Co., 35.58ºx-83.07º, coll. 31 May 2003 by C. Favret on *A. fraseri*. INHS411806, USA, NC, Haywood Co., 35.58ºx-83.07º, 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., 35.60ºx-83.46º, coll. 4 June 2003 by C. Favret on *A. fraseri*. INHS33636, USA, NC, Swain Co., 35.54ºx-83.49º, coll. 16 July 2003 by C. Favret on *A. fraseri*. INHS411803, USA, VT, WA Co., 44.08ºx-72.86º, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411803, USA, VT, WA Co., 44.08ºx-72.86º, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411816, USA, VT, Addison Co., 44.00ºx-73.02º, coll. 18 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411830, USA, VT, Addison Co., 43.94ºx-72.95º, coll. 19 June 2006 by C. Favret and S. Favret on *A. balsamea*. INHS411807, USA, VA, Grayson Co., 36.65ºx-81.58º, 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. INHS4363–48371, USA, MI, Kalamazoo Co., W. K. Kellogg Forest, coll. 11 June 1993 by D. J. Voegtlin on *A. grandis*. Males: INHS43835–48361, USA, MI, Kalamazoo Co., W. K. Kellogg Forest, coll. 6 May 1993 by R. Lawrence on *A. grandis*. INHS43863, INHS43865–48366, INHS43868,
INHS 48370, USA, MI, Kalamazoo Co., W. K. Kellogg Forest, coll. 11 June 1993 by D. J. Voegtlin on A. grandis.

Acknowledgments

David Voegtlin (INHS, Champaign, IL), Gary Miller (USDA Systematic Entomology Laboratory [SEL], Beltsville, MD), Stewart McKamey (SEL, Washington, DC), Susan Halbert (Florida Department of Agriculture and Consumer Services, Gainesville, FL), and an anonymous reviewer provided helpful comments on the manuscript. Paul Tinerella (INHS) loaned me the holotype of Schizoneura pinicola. Andrea Binazzi (Agricultural Research Council–Research Centre for Agrobiology and Pedology, Florence, Italy), Charlotte Nielsen (University of Copenhagen, Copenhagen, Denmark), David Voegtlin (INHS), and Ron Kelley (Vermont Department of Forests, Parks, and Recreation, Morrisville, VT) collected fresh specimens on behalf of the project.

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Received 15 March 2009; accepted 15 July 2009.