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RESEARCH ARTICLE

Distinguishing eastern North American forest moth pests by wing-scale ultrastructure: potential applications in paleoecology

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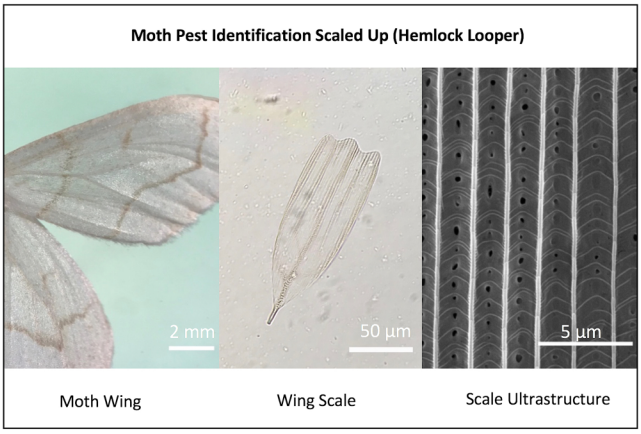
Abstract

The use of fossil moth wing scales has recently been introduced as a new method to reconstruct population histories of lepidopterans and provide a proxy for insect disturbance. We investigated the potential for using wing-scale ultrastructure to distinguish between the five most common outbreak species of moth pests in eastern North America: spruce budworm (*Choristoneura fumiferana* Clemens), hemlock looper (*Lambdina fiscellaria* Guenée), forest tent caterpillar (*Malacosoma disstria* Hübner), blackheaded budworm (*Acleris variana* Fernie), and jack pine budworm (*Choristoneura pinus* Freeman). Using scanning electron images of scales, we made qualitative and quantitative comparisons of morphological traits at the ultrastructural level. We found that hemlock looper and eastern blackheaded budworm scales could be categorically separated from each other and from the three other species. We developed a quadratic discriminant function using measurements of ultrastructure traits that distinguishes scales of the three remaining species with an overall accuracy of 66%. We found that forest tent caterpillar could be well separated based on these traits, but we were less confident in distinguishing the closely related jack pine and spruce budworm. Our method offers potential advantages in scale identification for future studies in paleoecology, while providing the additional advantage of not requiring intact, unfolded, and undamaged scales.



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Introduction

Insect defoliators are a major source of ecological disturbance in boreal forests across eastern North America (Volney 1996; Zhang et al. 2014). Canadian forests are home to at least 106 species of insect defoliators, with the order Lepidoptera contributing the greatest number (70%) and some of the most destructive forest pests due to defoliation of their hosts (Johns et al. 2016). Populations of these forest pests can increase to epidemic levels known as outbreaks. These outbreaks have generated a great deal of interest for economic and ecological reasons. Some species cause extensive damage to forests leading to severe reductions in the quality and abundance of harvestable timber resources. The economic impacts of these outbreaks can be significant. The eastern spruce budworm (*Choristoneura fumiferana* Clemens; Tortricidae) outbreak of 1967–1993 affected 50 million ha of forest in eastern North America (Sturtevant et al. 2015) with an estimated loss of 44 million m^3 of timber per year of the cycle. Economic models indicate potential losses of $10.8–15.3 billion in Atlantic Canada over the course of a cycle, depending on the severity of the outbreak (D.A. MacLean, unpublished data).

The population dynamics of spruce budworm have attracted special interest among ecologists because these populations cycle every 25–40 years over large areas. The factors that regulate and synchronize these cycles have been a long-standing interest among population ecologists (e.g., Swaine and Craighead 1924; Morris 1963; Blais 1983; Johns et al. 2016; Royama et al. 2017). The great length of spruce budworm cycles, however, means that relatively few cycles have been studied in detail and many of the interesting questions about the frequency of cycles—such as, how long have spruce budworm populations been cycling, what is the relationship between outbreak frequency and climate, has the geographical origin of outbreaks shifted through time—require long-term data necessitating a paleoecological approach (Pureswaran et al. 2016).

A number of direct and indirect paleoecological methods have been attempted for reconstructing the history of past outbreaks for a variety of forest insect pests. Dendrochronological techniques pioneered by Swaine and Craighead (1924) and Blais (1954) have been the most widely applied and most successful so far. The most popular method is based on a comparison of ring-widths of host and nonhost tree species of the target insect from a given site. The method, however, is limited by the age of the oldest trees available, which in the case of balsam fir, the preferred host of spruce budworm, and white pine, a commonly used nonhost species, is 300–400 years. Furthermore, the method is indirect and cannot distinguish other possible agents of ring-growth suppression, such as pathogens.

The most direct approach has been to recover fossil head capsules of spruce budworm larvae from sediments, although Davis et al. (1980) found that the capsules occurred too sporadically to provide reliable estimates of outbreak periodicity. Bhiry and Filion (1996) recovered head capsules of spruce budworm and hemlock looper (*Lambdina fiscellaria* Guenée; Geometridae) in association with insect-chewed hemlock needles dating from the mid-Holocene Hemlock Decline, but again, these head capsules were neither abundant nor consistently present from sample to sample. It is perhaps unsurprising that the direct study of head capsules in sediments has proven to be unrewarding because, unlike the abundant head capsules of chironomids whose larvae are aquatic, the terrestrial larvae of lepidopterans are unlikely to fall into lakes and become incorporated into sediments as fossils. Simard et al. (2002, 2006) devised the technique of using species-identifiable frass to determine past outbreaks of spruce budworm; however, the frass did not always preserve in peats, resulting in

fragmentary records. Thus, whereas some of the existing methods can produce useful century-scale records, no method has been able to produce detailed records of outbreaks on a millennial scale.

Recently, Navarro et al. (2018) introduced the novel use of moth wing scales to reconstruct past outbreaks of spruce budworm. Because wing scales are made of chitin, which is known to preserve well in lake sediments and peats, the technique offers the potential of producing detailed records of outbreaks over millennial time scales. Navarro et al. (2018) used linear discriminant analysis based on shape and other characteristics of wing scales as viewed by light microscopy to identify scales from spruce budworm, hemlock looper, and forest tent caterpillar moths (*Malacosoma disstria* Hübner; Lasiocampidae). Their analysis was able to correctly discriminate 68% of spruce budworm scales.

We have been independently developing the use of moth scales to reconstruct past outbreaks of forest moth pests. In this paper we examine the potential of using moth-scale ultrastructure to discriminate between the main species of moth pests of eastern North America. The taxonomic potential of wing-scale ultrastructure has been long recognized (Downey and Allyn 1975; Ghiradella 1984, 1991). Scanning electron microscopy (SEM) of moth-scale ultrastructure has been used to discriminate between families (Simonsen 2001; Simonsen and Kristensen 2001) and between species (Yang and Zhang 2011) of lepidopterans. Navarro et al.'s (2018) analysis included three species: spruce budworm, hemlock looper, and forest tent caterpillar. Our analysis builds upon those three species by including an additional two species: eastern blackheaded budworm (*Acleris variana* Fernie; Torticidae) and jack pine budworm (*Choristoneura pinus* Freeman; Torticidae). Additionally, we conducted analyses to determine if we can differentiate between male and female moth scales for each of the five species. Rhainds and Heard (2015) found that swarms formed during SBW outbreaks are female dominated. Thus, identification of predominately female scales in the fossil record could indicate the presence of swarms, typical during periods of high population densities. Our approach is to use SEM images of scale ultrastructure for qualitative morphological and quantitative morphometric comparisons between the five outbreak species.

Materials and methods

Specimens

We examined the wing-scale ultrastructure of the five most common moth pest species in eastern North America: eastern spruce budworm, hemlock looper, forest tent caterpillar, jack pine budworm, and eastern blackheaded budworm. Six males and six females of each species were examined.

Male spruce budworm moths were obtained from pheromone traps in Dalhousie, New Brunswick. Female spruce budworm, jack pine budworm, and blackheaded budworm moths were provided by the Great Lakes Forestry Centre, Sault Ste Marie, Ontario. Male jack pine budworm and blackheaded budworm specimens were caught in pheromone traps located in New Brunswick and Cape Breton, Nova Scotia, respectively. Hemlock looper specimens, both male and female were obtained from the Laurentian Forestry Centre, Quebec. The Forest Management branch of the Department of Energy and Resource Development, New Brunswick, donated forest tent caterpillar moths.

Preparation and examination of wing scales

We used forceps to scrape scales onto carbon-taped stubs separately from the dorsal side of a forewing and corresponding hindwing for each individual. The stub-mounted scales were then sputter-coated with gold for conductivity using an Edwards S150A coater. We collected secondary electron images of scales at a magnification of 5000× at the University of New Brunswick Microscopy and

Microanalysis Facility with a JEOL JSM-6400 Scanning Electron Microscope using an accelerating voltage of 15 kV. Images were acquired using a Digiscan II controlled by Gatan Digital Micrograph software.

Wing scale comparisons

Using terminology from Downey and Allyn (1975) and Ghiradella (1991), we first examined scales for differences in qualitative ultrastructure traits, including spacing of longitudinal ridges and cross ribs and occurrence of pores between longitudinal ridges (**Fig. 1**). We measured several ultrastructural traits for quantitative analysis. To determine the uniformity of cross-rib spacing, we counted the number of cross ribs within a selected 5 µm central section of the scale surface and measured the distance between them. Pores are a prominent feature of many scales. We counted the number of pores in a selected 5 µm section and measured five pores from that section for pore height (diameter parallel to longitudinal ridges), pore width (diameter perpendicular to longitudinal ridges), and calculated the area of individual pores using the formula: $A = \pi ab$, where a is the radius along the horizontal axis and b is the radius along the vertical axis of the pore. If pores were absent in the 5 µm section, but present elsewhere on the SEM image, measurements were taken for up to five randomly selected pores. We also measured the width of longitudinal ridges and distance between ridges. Measurements were made using imaging software Fiji version 1.0 (Schindelin et al. 2012).

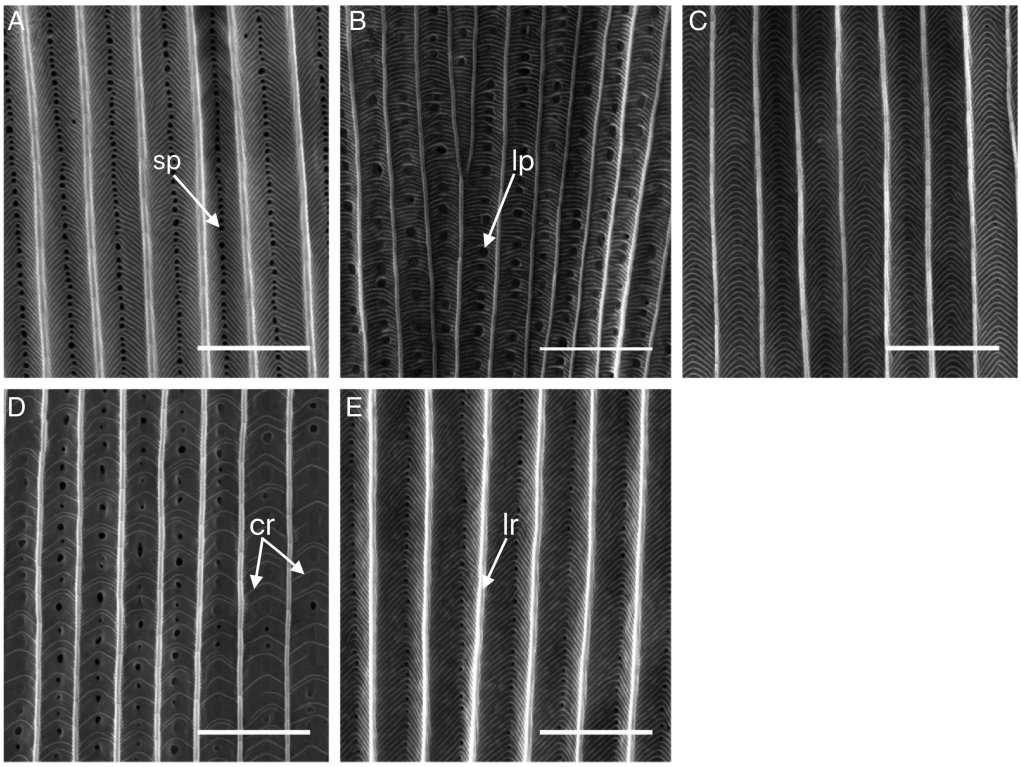

Fig. 1. Ultrastructure of wing scales of five moth outbreak species. (A) *Choristoneura pinus* (jack pine budworm), (B) *Malacosoma disstria* (forest tent caterpllar), (C) *Acleris variana* (eastern blackheaded budworm), (D) *Lambdina fiscellaria* (hemlock looper), and (E) *Choristoneura fumiferana* (spruce budworm). Ultrastructure traits include parallel longitudinal ridges (lr), cross ribs (cr), and both small (sp) and large (lp) pores. Scale bars = 5 µm.

Statistical analysis

Nonmetric multidimensional scaling

Clustering of scales and individual moths by wing scale ultrastructure was visualized by nonmetric multidimensional scaling (nMDS) using the metaMDS function from the vegan package (version 2.4-6; Oksanen et al. 2018). Dissimilarity matrices for the nMDS were based on Bray–Curtis distance measures.

Unique features for species identification

Two species (hemlock looper and blackheaded budworm) displayed unique scale features. Hemlock looper scales clearly displayed nonuniform spacing between cross ribs (**Fig. 1D**) and blackheaded budworm scales had few (if any) pores (**Fig. 1C**). To test statistically if nonuniform spacing between cross ribs could be used to distinguishing hemlock looper scales from the four other species, variance in the spacing between cross ribs per scale was analyzed using a linear mixed-effects model (LMM). Model parameters included fixed effects of species, sex, a species by sex interaction, and a random effect of moth (to account for repeated measures). Hemlock looper was used as the reference species for the conditional model of the fixed effects (i.e., treatment contrasts) and females were used as the reference sex. Conditional models allowed for comparisons in the variance in the distance between cross ribs of each individual species to hemlock looper. The "lmer" function from the "lme4" package (version 1.1-17; Bates et al. 2015) was used to conduct the LMM; uniformity of cross ribs was log transformed prior to analysis to achieve normality. Most blackheaded budworm scales had no pores, leading to an excessive number of zeros in the data. As such, a zero-inflated generalized linear mixed-effect model (ziGLMM) was used to determine if number of pores could be used to differentiate blackheaded budworm from the other species. The same fixed and random effects from the LMM were used as model parameters for the ziGLMM but treatment contrasts were done using blackheaded budworm as the reference species. Our ziGLMM was conducted using the "glmmTMB" function (family = gaussian) from the "glmmTMB" package (version 0.2.2.0; Brooks et al. 2017). The "drop1" function (package: "stats" version 3.4.0; R Core Team 2017) was used to evaluate the contribution of fixed effects in both univariate analyses. First the species by sex interaction was evaluated, when it was not significant we removed the interaction term and compared reduced models with one of the fixed effects removed to the full model with both effects present (following Zuur et al. 2009). Where the interaction was significant, we did not evaluate the main effects independently.

Discriminant analysis

No unique features of scale ultrastructure were identified to differentiate among forest tent caterpillar, jack pine budworm, and spruce budworm. Therefore, we applied a discriminant analysis (DA) to determine whether ultrastructure could be used to differentiate wing scales of these three species. DA requires independent observations (Zuur et al. 2007). Because we had repeated measures (multiple scales per moth), which violate assumptions of independence, we pooled values and reported each trait as the mean value per individual moth (**Supplementary Material 1**). Given our small sample size (six of each sex per species) and high degree of overlap for female and male forest tent caterpillar and spruce budworm (**Fig. 2**) we did not include sex as a grouping factor in our DA (i.e., we used the DA to group individuals by species only).

An underlying assumption of DA is that the variables used to discriminate among groups follow a multivariate normal distribution. Although normality of the individual traits does not ensure a multivariate normal distribution, it can be used as a reasonable first step in achieving multivariate normality (Looney 1995). As such, each trait was visually assessed to determine if it generally followed a normal distribution. Traits deviating from normality (e.g., skewed or bimodal distributions) even after normalizing transformations were applied (i.e., log, arcsine square root transforms) were not included in the DA. Four candidate traits were identified: number of cross ribs, distance between longitudinal ridges,

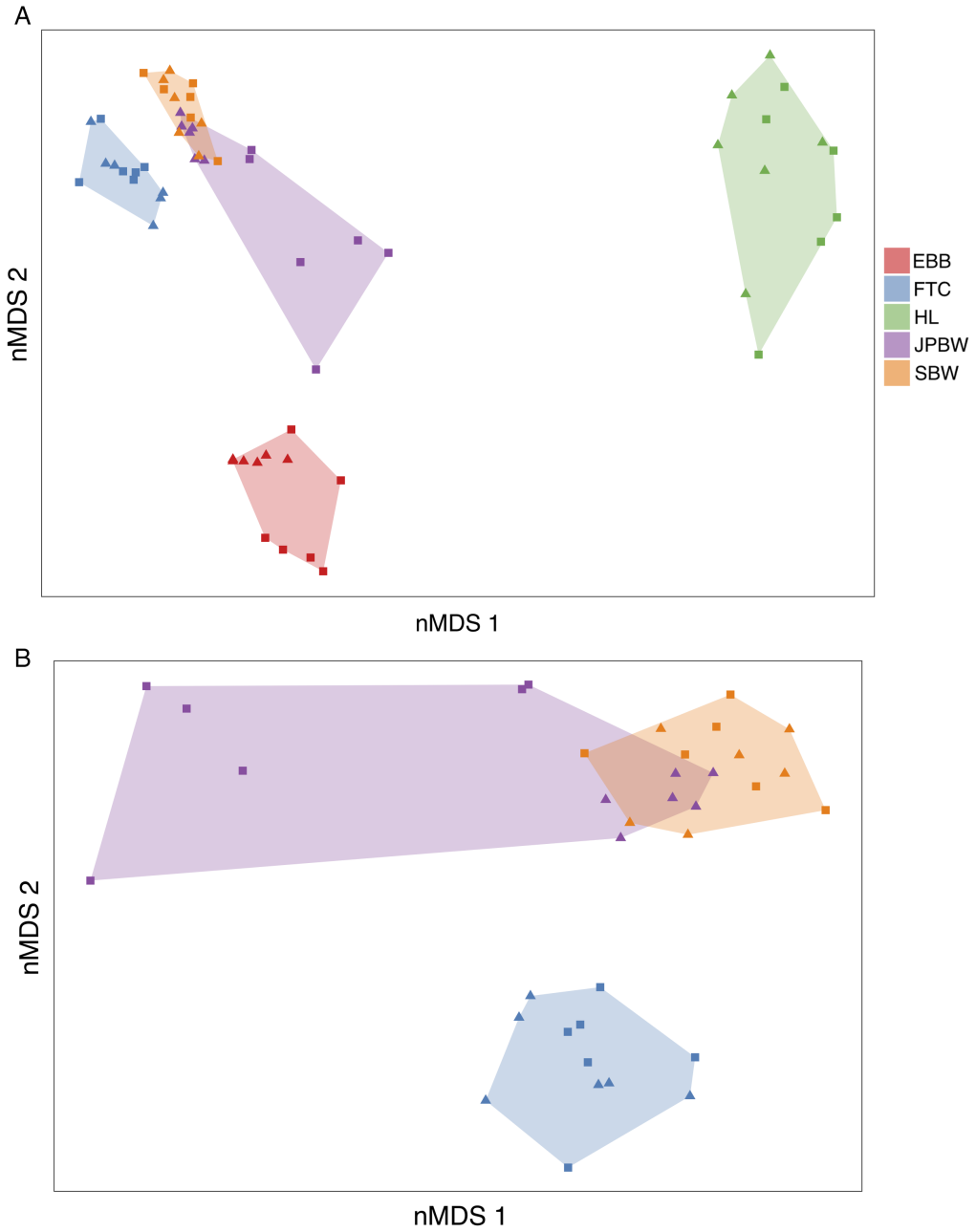

Fig. 2. Nonmetric multidimensional scaling (nMDS) plot presenting the differences in wing-scale ultrastructure among species for (A) all measured traits and species of moths studied, *Choristoneura pinus* (jack pine budworm, JPBW), *Malacosoma disstria* (forest tent caterpillar, FTC), *Acleris variana* (eastern blackheaded budworm, EBB), *Lambdina fiscellaria* (hemlock looper, HL), and *Choristoneura fumiferana* (spruce budworm, SBW) and (B) the three species of moths and selected traits used in the quadratic discriminant analysis (JPBW, FTC, and SBW) (see text for details). Squares represent female moths and triangles represent males.

width of longitudinal ridges, and the number of pores. With these four candidate traits we formally tested all the underlying assumptions of DA. The only assumption not met was homogeneity of variance. We therefore used a quadratic discriminant analysis (QDA) over a linear discriminant analysis as QDA does not have an underlying assumption of homogeneity (Zuur et al. 2007).

Training and testing the QDA

Seventy percent of our data was randomly selected and used to train our model ($n = 25$) and the remaining 30% was used to test its accuracy ($n = 11$). Since our goal was to identify species from single scales, we also randomly selected one-third ($n = 120$) of the individual scales (i.e., not pooled values per moth) to test our model's predictive ability at the scale level. The process of randomly selecting training data, moth-level test data, and scale-level test data was bootstrapped 100 times. For each bootstrap we reported the accuracy (percent correct identifications) for the training and moth-level test data. For the scale-level test data, we reported the percent of correct identifications per species and the percent of scales misidentified as either of the two additional species (e.g., percent of spruce budworm scales identified as forest tent caterpillar, percent spruce budworm identified as jack pine budworm, etc.). Our QDA was conducted using the "qda" function from the "MASS" package (version 7.3-49; Venables and Ripley 2002). All statistical analyses were conducted using R software (version 4.4.0; R Core Team 2017).

Results

Cross-rib variance

Variance in the distance between cross ribs was highest in hemlock looper; all other species had relatively uniform spacing with little variance (Fig. 3). The LMM revealed no statistically significant interactions between species and sex, nor was there a significant effect of sex on cross-rib variance ($F = 2.15$, df = 1, $p = 0.15$ and $F = 2.00$, df = 4, $p = 0.11$, respectively). However, there was a significant

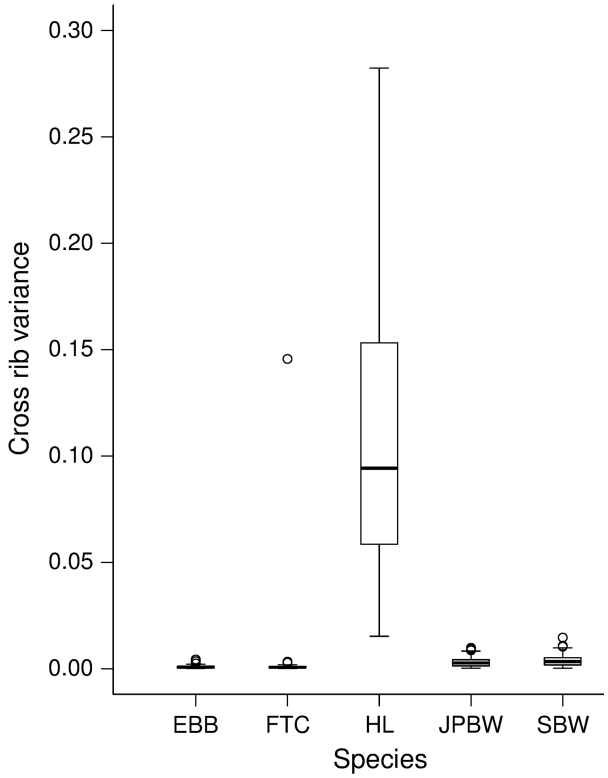

Fig. 3. Box and whisker plots showing variance in the distance between cross ribs per scale for each species of moth, *Choristoneura pinus* (jack pine budworm, JPBW), *Malacosoma disstria* (forest tent caterpillar, FTC), *Acleris variana* (eastern blackheaded budworm, EBB), *Lambdina fiscellaria* (hemlock looper, HL), and *Choristoneura fumiferana* (spruce budworm, SBW). Boxes represent 25% and 75% quartiles and the middle line represent the median. Whiskers show 2.5% and 97.5% quantiles and points depict any outliers. One extreme outlier for HL (cross-rib variance = 0.80) was not included in the figure.

Table 1. Treatment contrast results for the main effect of species from our linear mixed-effect model testing the effect of moth species and sex on the variance among cross ribs per scale for *Choristoneura pinus* (jack pine budworm, JPBW), *Malacosoma disstria* (forest tent caterpillar, FTC), *Acleris variana* (eastern blackheaded budworm, EBB), *Lambdina fiscellaria* (hemlock looper, HL), and *Choristoneura fumiferana* (spruce budworm, SBW).

Parameters	Estimate (SE)	*t* value	*p*
(Intercept)	−2.23 (0.12)	−18.1	**<0.01**
EBB	−4.99 (0.17)	−28.7	**<0.01**
FTC	−5.11 (0.17)	−29.3	**<0.01**
JPBW	−3.94 (0.17)	−22.6	**<0.01**
SBW	−3.36 (0.17)	−19.3	**<0.01**
Sex (M)	−0.30 (0.17)	−1.72	0.09
EBB: sex (M)	0.25 (0.25)	1.03	0.31
FTC: sex (M)	0.37 (0.25)	1.50	0.14
JPBW: sex (M)	0.43 (0.25)	1.74	0.09
SBW: sex (M)	−0.15 (0.25)	−0.60	0.55

Note: Hemlock looper was used as the reference species for species contrasts.

main effect of species ($F = 495$, df = 4, $p < 0.001$). Treatment contrasts revealed significantly lower variance in cross-rib spacing for each individual species as compared with hemlock looper (Table 1).

Number of pores

Blackheaded budworm had the lowest number of pores per scale (Fig. 4). However, there was significant species by sex interaction (LRT = 18.73, df = 4, $p < 0.001$). Closer inspection revealed that the species by sex interaction is driven by the difference between male and female jack pine budworm (Fig. 4; Table 2). Because this interaction does not affect blackheaded budworm (Table 2) and the 95% confidence intervals from the scales of either sex of jack pine budworm do not overlap with blackheaded budworm (mean 4.73 ± 1.81 and 7.90 ± 0.51 for female and male jack pine budworm, respectively, and 0.13 ± 0.11 for blackheaded budworm), we felt it was appropriate to interpret treatment contrasts (among species) to test our assumption that blackheaded budworm has fewer pores compared to the other species. Contrasts were all positive and significant (Table 2), indicating that blackheaded budworm had significantly fewer pores than each of the other four species.

Quadratic discriminant analysis (QDA)

Mean (± SD) accuracy rates of the bootstrapped QDA were 92% ± 4% for the training data, 79% ± 12% for moth-level test data, and 66% ± 6% for scale test data. Forest tent caterpillar and jack pine budworm scales were more likely to be correctly identified than spruce budworm (Table 3). We found good separation of forest tent caterpillar from the budworm species (jack pine budworm and spruce budworm); an average of 24% of forest tent caterpillar scales are incorrectly identified as either budworm species (Table 3). Most misidentifications were between the two species of budworm. It is rare for a budworm scale to be identified as forest tent caterpillar (an average of 8% of scales from each species are identified as forest tent caterpillar); however, differentiating between the two budworm species is more difficult and where most of the misidentifications occur (Table 3).

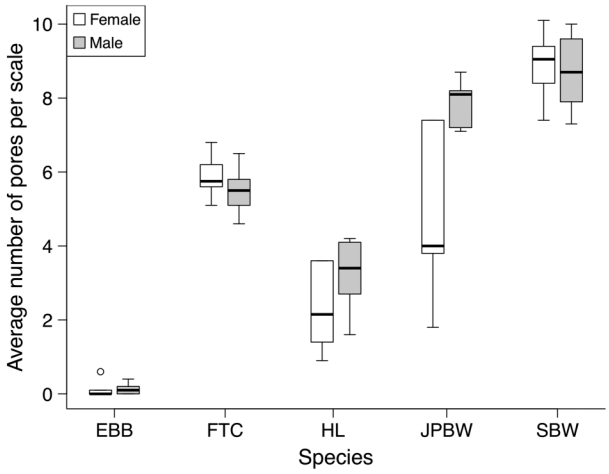

Fig. 4. Box and whisker plots showing number average number of pores per scale for both female and male moths of each species, *Choristoneura pinus* (jack pine budworm, JPBW), *Malacosoma disstria* (forest tent caterpillar, FTC), *Acleris variana* (eastern blackheaded budworm, EBB), *Lambdina fiscellaria* (hemlock looper, HL), and *Choristoneura fumiferana* (spruce budworm, SBW). Boxes represent 25% and 75% quartiles and the middle line represent the median. Whiskers show 2.5% and 97.5% quantiles and the open circle points depict the outliers.

Table 2. Treatment contrast results for the main effect of species from our general linear mixed-effect model testing the effect of moth species and sex on the number of pores per scale for *Choristoneura pinus* (jack pine budworm, JPBW), *Malacosoma disstria* (forest tent caterpillar, FTC), *Acleris variana* (eastern blackheaded budworm, EBB), *Lambdina fiscellaria* (hemlock looper, HL), and *Choristoneura fumiferana* (spruce budworm, SBW).

Parameters	Estimate (SE)	*z* value	*p*
(Intercept)	0.30 (0.50)	0.60	0.55
FTC	5.57 (0.06)	9.31	<0.01
HL	4.19 (0.73)	5.78	<0.01
JPBW	5.43 (0.62)	8.77	<0.01
SBW	8.60 (0.60)	14.38	<0.01
Sex (M)	0.03 (0.70)	0.04	0.97
FTC: sex (M)	−0.39 (0.84)	−0.47	0.64
HL: sex (M)	0.42 (0.97)	0.43	0.67
JPBW: sex (M)	2.42 (0.86)	2.83	<0.01
SBW: sex (M)	−0.23 (0.84)	−0.27	0.79

Note: Blackheaded budworm was used as the reference species.

Discussion

The purpose of the methods developed in this paper and that of Navarro et al. (2018) is to identify fossil moth scales to species in an effort to reconstruct the outbreak histories of specific forest moth pests. Fossil Lepidoptera wing scales provide direct evidence of the past occurrence of moth species and are a promising new paleoecological proxy to reconstruct past insect disturbance (Montoro

Table 3. Mean accuracy rates of scale identification (proportion correct) on the diagonal, with mean proportion misidentified on the off-diagonal for three species: *Choristoneura pinus* (jack pine budworm JPBW), *Malacosoma disstria* (forest tent caterpillar FTC), and *Choristoneura fumiferana* (spruce budworm SBW).

	Predicted		
Actual	**FTC**	**JPBW**	**SBW**
FTC	**0.76**	0.17	0.07
JPBW	0.08	**0.69**	0.23
SBW	0.08	0.39	**0.53**

Note: Values represent mean accuracy from quadratic discriminant analysis with bootstrapped training and test data (see text for details).

Girona et al. 2018; Navarro et al. 2018). The use of scale ultrastructure to identify fossil scales is an important contribution to this growing field.

We showed that blackheaded budworm and hemlock looper can be categorically separated from forest tent caterpillar, jack pine budworm, and spruce budworm based on unique qualitative morphological traits of scale ultrastructure. This result suggests that distinctive morphological traits could be used to identify unknown fossil moth scales of hemlock looper and blackheaded budworm. Blackheaded budworm was included in our analysis because it shares the same preferred host species as spruce budworm and, historically, outbreaks of the two species have overlapped both spatially and temporally, as during their outbreaks in the 1940s in eastern Canada (Miller 1966). It is therefore important to be able to separate these two taxa. Also, although Navarro et al.'s (2018) analysis correctly classified a high proportion of hemlock looper scales (79%), we determined that hemlock looper can be categorically separated from the other four major moth outbreak species based on scale ultrastructure, without the need for DA.

QDA showed that forest tent caterpillar was well separated from the budworm species (jack pine budworm and spruce budworm), with only 24% of forest tent caterpillar scales incorrectly assigned to either jack pine budworm or spruce budworm. We found that most misidentifications were between spruce budworm and jack pine budworm (39% of spruce budworm incorrectly assigned as jack pine budworm, and 23% jack pine budworm incorrectly assigned as spruce budworm), indicating a high degree of similarity in scale ultrastructure between these two species. Spruce budworm and jack pine budworm are closely related species both belonging to the genus *Choristoneura* (Freeman 1967), whose scales we correctly distinguished from forest tent caterpillar 76% of the time. The hosts and outbreak characteristics of spruce budworm and jack pine budworm, however, differ. Jack pine budworms have a strong preference for jack pine (*Pinus banksiana* Lambert) (Volney 1988) but, larvae will occasionally feed on other pine species (Kulman and Hodson 1961) as opposed to spruce budworm, whose hosts are chiefly balsam fir (*Abies balsamea* (L.) Miller), white spruce (*Picea glauca* (Moench) Voss), and black spruce (*Picea mariana* Miller). Jack pine stands are found primarily on dry, sandy soils. Given the strong preference jack pine budworm has for its host and its host for particular edaphic conditions, study sites could be selected to minimize the past local occurrence of jack pine trees and therefore be able to presume that any *Choristoneura* scale found is likely from spruce budworm.

Fossils, such as wing scales, are commonly found in suboptimal condition. The method developed by Navarro et al. (2018) differs from ours in that scale morphometrics can only be measured on entire scales, excluding any scales that were broken or folded. A potential advantage of our method when

applied as a paleoecological tool is that scale ultrastructure can be observed as long as a central fragment of a scale is present. If fossils are damaged or folded, our method provides the advantage of being able to identify a greater proportion of fossil scales towards establishing a reasonable count; a significant advantage if scales are not especially abundant at a site.

Conclusion

Of the five most common outbreaking moth species in eastern North America, hemlock looper and blackheaded budworm can be categorically identified by qualitative differences in scale ultrastructure. Although we observed overlap between the two *Choristoneura* sp. in both the nMDS and QDA results, good separation was shown between forest tent caterpillar and these two species based on differences in scale ultrastructure traits. We conclude from our study that scale ultrastructure can be used to distinguish hemlock looper and blackheaded budworm categorically, and forest tent caterpillar with a high degree of certainty using QDA. Identification of the two remaining species (jack pine budworm and spruce budworm) is more difficult due to overlap of scale ultrastructure traits, although site selection to maximize for spruce budworm habitat and minimize jack pine budworm habitat may improve the assignment of fossil scales of *Choristoneura* to species.

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Author contributions

LCC conceived and designed the study. KJM performed the experiments/collected the data. KJM, LCC, and SE analyzed and interpreted the data. LCC contributed resources. KJM, LCC, and SE drafted or revised the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Data availability statement

All relevant data are within the paper and the supplementary material.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2018-0051.

Supplementary Material 1

References

Bates D, Mächler M, Bolker B, and Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67: 1–48. DOI: 10.18637/jss.v067.i01

Bhiry N, and Filion L. 1996. Mid-Holocene hemlock decline in eastern North America linked with phytophagous insect activity. Quaternary Research, 45: 312–320. DOI: 10.1006/qres.1996.0032

Blais JR. 1954. The recurrence of spruce budworm infestations in the past century in the Lac Seul area of northwestern Ontario. Ecology, 35: 62–71. DOI: 10.2307/1931405

Blais JR. 1983. Trends in the frequency, extent, and severity of spruce budworm outbreaks in eastern Canada. Canadian Journal of Forest Research, 13: 539–547. DOI: 10.1139/x83-079

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal, 9: 378–400. DOI: 10.32614/RJ-2017-066

Davis RB, Anderson RS, and Hoskins BR. 1980. A new parameter for paleoecological reconstruction: head capsules of forest-tree defoliator Microlepidopterans in lake sediment. *In* Abstracts and Program of the 6th Biennial Meeting of the American Quaternary Association, Orono, Maine, 18–20 August 1980. Institute of Quaternary Studies, University of Maine, Orono, Maine. p. 62.

Downey JC, and Allyn AC. 1975. Wing-scale morphology and nomenclature. Bulletin of Allyn Museum, 31: 1–32.

Freeman TN. 1967. On coniferophagous species of *Choristoneura* (Lepidoptera: Tortricidae) in North America: I. Some new forms of *Choristoneura* allied to *C. fumiferana*. The Canadian Entomologist, 99: 449–455. DOI: 10.4039/Ent99449-5

Ghiradella H. 1984. Structure of iridescent Lepidopteran scales: variations on several themes. Annals of the Entomological Society of America, 77: 637–645. DOI: 10.1093/aesa/77.6.637

Ghiradella H. 1991. Light and color on the wing: structural colors in butterflies and moths. Applied Optics, 30: 3492–3500. PMID: 20706416 DOI: 10.1364/AO.30.003492

Johns RC, Flaherty L, Carleton D, Edwards S, Morrison A, and Owens E. 2016. Population studies of tree-defoliating insects in Canada: a century in review. The Canadian Entomologist, 148: S58–S81. DOI: 10.4039/tce.2015.69

Kulman HM, and Hodson AC. 1961. Parasites of the jack-pine budworm, *Choristoneura pinus*, with special reference to parasitism at particular stand locations. Journal of Economic Entomology, 54: 221–224. DOI: 10.1093/jee/54.2.221

Looney SW. 1995. How to use tests for univariate normality to assess multivariate normality. The American Statistician, 49: 64–70. DOI: 10.2307/2684816

Miller CA. 1966. The black-headed budworm in eastern Canada. The Canadian Entomologist, 98: 592–613. DOI: 10.4039/Ent98592-6

Montoro Girona M, Navarro L, and Morin H. 2018. A secret hidden in the sediments: Lepidoptera scales. Frontiers in Ecology and Evolution, 6: 1–5. DOI: 10.3389/fevo.2018.00002

Morris RF. 1963. The dynamics of epidemic spruce budworm populations. The Memoirs of the Entomological Society of Canada, 95: 1–12. DOI: 10.4039/entm9531fv

Navarro L, Harvey A, and Morin H. 2018. Lepidoptera wing scales: a new paleoecological indicator for reconstructing spruce budworm abundance. Canadian Journal of Forest Research, 48: 302–308. DOI: 10.1139/cjfr-2017-0009

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. 2018. vegan: community ecology package. R package version 2.4-6 [online]: Available from cran.r-project.org/web/packages/vegan/index.html.

Pureswaran DS, Johns R, Heard SB, and Quiring D. 2016. Paradigms in eastern spruce budworm (Lepidoptera: Tortricidae) population ecology: a century of debate. Environmental Entomology, 45: 1333–1342. PMID: 28028079 DOI: 10.1093/ee/nvw103

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [online]: Available from r-project.org/.

Rhainds M, and Heard SB. 2015. Sampling procedures and adult sex ratios in spruce budworm. Entomologia Experimentalis et Applicata, 154: 91–101. DOI: 10.1111/eea.12257

Royama T, Eveleigh ES, Morin JRB, Pollock SJ, McCarthy PC, McDougall GA, et al. 2017. Mechanisms underlying spruce budworm outbreak processes as elucidated by a 14-year study in New Brunswick, Canada. Ecological Monographs, 87: 600–631. DOI: 10.1002/ecm.1270

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods, 9: 676–682. PMID: 22743772 DOI: 10.1038/nmeth.2019

Simard I, Morin H, and Potelle B. 2002. A new paleoecological approach to reconstruct long-term history of spruce budworm outbreaks. Canadian Journal of Forest Research, 32: 428–438. DOI: 10.1139/x01-215

Simard I, Morin H, and Lavoie C. 2006. A millennial-scale reconstruction of spruce budworm abundance in Saguenay, Québec, Canada. The Holocene, 16: 31–37. DOI: 10.1191/0959683606hl904rp

Simonsen TJ. 2001. The wing vestiture of the non-ditrysian Lepidoptera (Insecta). Comparative morphology and phylogenetic implications. Acta Zoologica, 82: 275–298. DOI: 10.1046/j.1463-6395.2001.00089.x

Simonsen TJ, and Kristensen NP. 2001. Agathiphaga wing vestiture revisited: evidence for complex early evolution of lepidopteran scales (Lepidoptera: Agathiphagidae). Insect Systematics & Evolution, 32: 169–175. DOI: 10.1163/187631201X00128

Sturtevant BR, Cooke BJ, Kneeshaw DD, and MacLean DA. 2015. Modeling insect disturbance across forested landscapes: insights from the spruce budworm. *In* Modeling forest landscape disturbances. *Edited by* A Perera, BR Sturtevant, and L Buse. Springer, New York, New York. pp. 93–134.

Swaine JM, and Craighead FC. 1924. Studies on the spruce budworm (Cacoecia fumiferana Clem.). Part I. A general account of the outbreaks, injury and associated insects. Bulletin No. 37—new series (technical). Dominion of Canada, Department of Agriculture, Ottawa, Ontario.

Venables WN, and Ripley BD. 2002. Modern applied statistics with S. 4th edition. Springer, New York, New York. ISBN 0-387-95457-0.

Volney WJA. 1988. Analysis of historic jack pine budworm outbreaks in the Prairie provinces of Canada. Canadian Journal of Forest Research, 18: 1152–1158. DOI: 10.1139/x88-177

Volney WJA. 1996. Climate change and management of insect defoliators in boreal forest ecosystems. *In* Forest ecosystems, forest management and the global carbon cycle. NATO ASI Series (Series I: Global Environmental Change). *Edited by* MJ Apps and DT Price. Springer, Berlin, Heidelberg, Germany. pp. 79–87.

Yang Z, and Zhang Y. 2011. Comparison of ultrastructure among sibling species of *Ostrinia* (Lepidoptera: Crambidae) from China. The Canadian Entomologist, 143: 126–135. DOI: 10.4039/n10-049

Zhang X, Lei Y, Ma Z, Kneeshaw D, and Peng C. 2014. Insect-induced tree mortality of boreal forests in eastern Canada under a changing climate. Ecology and Evolution, 4: 2384–2394. PMID: 25360275 DOI: 10.1002/ece3.988

Zuur AF, Ieno EN, and Smith GM. 2007. Analyzing ecological data. Springer, New York, New York. DOI: 10.1007/978-0-387-45972-1

Zuur AF, Ieno EN, Walker N, Saveliev AA, and Smith GM. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, New York.