Identification of cocoons of *Apanteles* and *Dolichogenidea* (Hymenoptera: Braconidae) species attacking *Choristoneura fumiferana* (Lepidoptera: Tortricidae) and associated Microlepidoptera

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The Canadian Entomologist **135**: 677 – 684 (2003)

Abstract—We examined the cocoons of six species of the genera *Apanteles* and *Dolichogenidea* attacking spruce budworm, *Choristoneura fumiferana* Clemens, and Microlepidoptera in the same microhabitat in an effort to overcome taxonomic and ecological problems associated with the identification of these species when adults fail to emerge from their cocoons. Neither cocoon length nor width nor ratio of length to width could be used to identify the six species, owing to considerable overlap in these attributes among the species and the effects of the source of the cocoons. Using a simple technique to examine webbing characteristics of the cocoons, however, we found that each species has a unique banding pattern, determined by the manner in which the density of the webbing varies along the length of the cocoon. This pattern can be used to reliably identify each species. We describe and illustrate the webbing characteristics of each species and provide an identification key based on these characteristics.

Eveleigh ES, Pollock SJ, Goulet H, Huber J. 2003. Identification des cocons des espèces *Apanteles* et *Dolichogenidea* (Hymenoptera : Braconidae) s'attaquant à *Choristoneura fumiferana* (Lepidoptera : Tortricidae) et aux Microlépidoptères apparentés. *The Canadian Entomologist* **135** : 677–684.

Résumé—Nous avons examiné les cocons de six espèces des genres *Apanteles* et *Dolichogenidea* qui s'attaquent à la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* Clemens, et aux microlépidoptères apparentés afin d'élucider les problèmes taxonomiques et écologiques, inhérents à l'identification de ces espèces lorsque les adultes n'arrivent pas à sortir de leur cocon. Ni la longueur du cocon, ni

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sa largeur, ni le rapport longueur à largeur n'ont pu être utilisés pour identifier les six espèces, du fait du chevauchement considérable de ces paramètres d'une espèce à l'autre, et des effets exercés par la source des cocons. En employant une technique simple pour examiner les caractéristiques de tissage du cocon, nous avons constaté que chaque espèce présente un mode unique d'enroulement, déterminé par la façon dont la densité de tissage varie sur la longueur du cocon. Ce mode d'enroulement permet de caractériser avec fiabilité chaque espèce. Nous décrivons et illustrons les caractéristiques de tissage des diverses espèces et proposons une clé permettant d'identifier chaque espèce selon ces caractéristiques.

Introduction

Larvae of spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), and of various other species of Microlepidoptera associated with the spruce budworm microhabitat, are reported to be attacked by four parasitoid species belonging to the genus *Apanteles* (*A. fumiferanae* Viereck, *A. milleri* Mason, *A. morrisi* Mason, and *A. petrovae* Walley) (Hymenoptera: Braconidae) and two parasitoid species belonging to the genus *Dolichogenidea* (*D. absona* (Muesebeck) and *D. renaulti* Mason) (Hymenoptera: Braconidae) (Miller and Renault 1976; Huber *et al.* 1996). Initially, all six species were placed in the genus *Apanteles* (Mason 1974), but Mason (1981) later transferred two of the species into the genus *Dolichogenidea*. Current taxonomic keys for species identification of both sexes are based solely on adult characteristics (Mason 1974).

During our studies in New Brunswick, Canada, on the population ecology of the spruce budworm, we recovered all six parasitoid species from host rearings (ES Eveleigh, unpublished data). Our efforts, however, to determine the incidence of larval parasitism by each of these parasitoid species were hampered because many specimens died in their cocoons, making it impossible to identify the parasitoids using either the currently available taxonomic keys or the original species descriptions. For the same reason, the identity and, hence, incidence of hyperparasitism and predation of these parasitoids from field-collected cocoons could not be properly estimated. Cocoons of all six species can occur concurrently in the field and all appear similar morphologically.

Recognizing the need to overcome the taxonomic problem caused when adults fail to emerge from their cocoons, we undertook a study of the cocoon characteristics of each species. We found that, although the cocoons of all species are white and opaque, each species has a unique pattern of fibers laid down along the length of the cocoon, as described herewithin.

Materials and methods

All cocoons examined were collected from two plots located approximately 11 km apart at the Acadia Research Forest (46°00′N, 66°25′W) near Fredericton, New Brunswick. Cocoons of each species were obtained from one or more of the following four sources: (1) laboratory rearing of wild spruce budworm larvae collected from the plots; (2) laboratory rearing of laboratory stock spruce budworm larvae obtained from the Canadian Forest Service, Sault Ste. Marie, Ontario, that had been implanted on host trees in the field for a period of time; (3) laboratory rearing of cocoons that issued in the field mainly from wild spruce budworm; and (4) laboratory rearing of cocoons that issued in the field mainly from implanted laboratory stock spruce budworm. In contrast to the first two sources, the host species from which the field-collected parasitoids emerged in the latter two sources cannot be determined, and some parasitoids may have emerged from associated microlepidopteran larvae. Undoubtedly, this is the case for

TABLE 1. Length (mm), width (mm), and ratio of length to width of the cocoons of six species of the genera *Apanteles* and *Dolichogenidea* attacking *Choristoneura fumiferana* and Microlepidoptera in the same microhabitat (data from all sources of cocoons combined for each species).

Species	Sex	*n*	Length (mean ± SD)	Width (mean ± SD)	Ratio (mean ± SD)
Apanteles fumiferanae	Male	50	3.72±0.21 (3.28–4.08)	1.35±0.10 (1.20–1.52)	2.76±0.12 (2.53–3.00)
	Female	50	3.99±0.27 (3.44–4.56)	1.43±0.12 (1.20–1.68)	2.81±0.11 (2.52–3.00)
Apanteles petrovae	Male	36	3.81±0.26 (3.20–4.40)	1.37±0.08 (1.20–1.52)	2.78±0.15 (2.35–3.06)
	Female	30	4.24±0.26 (3.68–4.80)	1.40±0.06 (1.20–1.52)	3.03±0.12 (2.71–3.33)
Apanteles morrisi	Male	30	4.23±0.17 (3.84–4.48)	1.46±0.06 (1.36–1.60)	2.89±0.09 (2.74–3.06)
	Female	30	4.43±0.14 (4.08–4.64)	1.49±0.07 (1.28–1.60)	2.98±0.08 (2.80–3.19)
Apanteles milleri	Male	2	3.84±0.23 (3.68–4.00)	1.24±0.06 (1.20–1.28)	3.10±0.04 (3.07–3.13)
	Female	1	4.32	1.36	3.18
Dolichogenidea absona	Male	85	3.65±0.27 (3.04–4.40)	1.22±0.08 (0.96–1.36)	2.99±0.15 (2.65–3.36)
	Female	55	4.40±0.30 (3.68–5.04)	1.29±0.09 (1.12–1.44)	3.41±0.20 (3.00–3.93)
Dolichogenidea renaulti	Male	—	—	—	—
	Female	5	4.70±0.15 (4.48–4.88)	1.49±0.04 (1.44–1.52)	3.16±0.13 (2.95–3.28)

NOTE: Ranges are in parentheses in the last three columns.

TABLE 2. ANOVA summary for the effects of source of cocoons and sex of cocoons on the length, width, and ratio of length to width of *Apanteles fumiferanae*, *Apanteles petrovae*, *Apanteles morrisi*, and *Dolichogenidea absona* cocoons.

Parasitoid species and variable	Source of variation	df	Mean square	*F*	*P*
Apanteles fumiferanae					
Length	Source of cocoons*	1	3.84154	196.08	0.0001
	Sex	1	1.90745	97.36	0.0001
	Source × sex	1	0.04532	2.31	0.1316
	Error	95	0.01959		
Width	Source of cocoons*	1	0.73533	156.11	0.0001
	Sex	1	0.15063	31.98	0.0001
	Source × sex	1	0.00177	0.38	0.5409
	Error	95	0.00471		
Ratio	Source of cocoons*	1	0.09140	7.59	0.0070
	Sex	1	0.04752	3.95	0.0498
	Source × sex	1	0.00465	0.39	0.5357
	Error	95	0.01204		
Apanteles petrovae					
Length	Source of cocoons†	1	0.88023	13.76	0.0005
	Sex	1	1.90384	29.76	0.0001
	Source × sex	1	0.01802	0.28	0.5979
	Error	51	0.06398		
Width	Source of cocoons†	1	0.01956	4.18	0.0461
	Sex	1	0.00199	0.43	0.5173
	Source × sex	1	0.00347	0.74	0.3933
	Error	51	0.00468		
Ratio	Source of cocoons†	1	0.15382	9.58	0.0032
	Sex	1	0.79191	49.33	0.0001
	Source × sex	1	0.04509	2.81	0.0999
	Error	51	0.01605		
Apanteles morrisi					
Length	Source of cocoons†	1	0.17306	9.35	0.0035
	Sex	1	0.54008	29.17	0.0001
	Source × sex	1	0.02993	1.62	0.2093
	Error	52	0.01852		
Width	Source of cocoons†	1	0.01712	4.36	0.0418
	Sex	1	0.00803	2.04	0.1589
	Source × sex	1	0.00037	0.09	0.7592
	Error	52	0.00393		
Ratio	Source of cocoons†	1	0.00084	0.12	0.7254
	Sex	1	0.10601	15.69	0.0002
	Source × sex	1	0.00726	1.07	0.3048
	Error	52	0.00676		
Dolichogenidea absona					
Length	Source of cocoons‡	2	2.45276	43.44	<0.0001
	Sex	1	16.6218	294.40	<0.0001
	Source × sex	2	0.18632	3.30	0.0399
	Error	133	0.05646		
Width	Source of cocoons‡	2	0.15536	27.36	<0.0001
	Sex	1	0.13147	23.15	<0.0001

https://doi.org/10.4039/n03-023 Published online by Cambridge University Press

TABLE 2 (*concluded*).

Parasitoid species and variable	Source of variation	df	Mean square	*F*	*P*
	Source × sex	2	0.00619	1.09	0.3392
	Error	133	0.00568		
Ratio	Source of cocoons‡	2	0.11565	3.97	0.0211
	Sex	1	5.44926	187.28	<0.0001
	Source × sex	2	0.11751	4.04	0.0198
	Error	133	0.02910		

* Cocoon sources 1 and 3 (see Materials and methods for details).
† Cocoon sources 2 and 4 (see Materials and methods for details).
‡ Cocoon sources 1, 2, and 4 (see Materials and methods for details).

most *D. renaulti* cocoons examined, because we failed to obtain this species from many thousands of spruce budworm larvae reared annually in the laboratory. To ensure that the cocoons examined were accurately identified, we examined only those cocoons from which adults had emerged and had been subsequently identified using Mason's (1974) key to species.

Cocoon length (mm) (including cap) and cocoon width (mm) at mid-length were measured using a dissecting microscope fitted with an ocular scale.

Webbing characteristics of empty cocoons (*i.e.*, those from which adults eclosed) were examined by holding the cocoons with soft forceps in front of a bright light source, such as a desk lamp or ceiling light, and viewing the intensity of light penetration throughout the cocoons. Areas of the cocoons where fibers are less dense permit more light to penetrate, thus allowing banding patterns to be observed. Hand drawings were made of the patterns observed for each species to determine whether patterns were consistent within and between species. Later, photographs were taken of typical cocoon patterns for each parasitoid species using a Lumina® camera attached to a Nikon® stereoscope.

Results

Measurements

There was considerable overlap in the range of length, width, and ratio of length to width measurements among the cocoons formed by males and females of the six parasitoid species (Table 1). For the four species with adequate data for analyses, the length and width of the cocoons differed significantly among the sources of the cocoons (Table 2). The ratio variable differed significantly among the sources of cocoons in all species except *A. morrisi* (Table 2). The length and ratio variables differed significantly between the sexes in all species, but the width of cocoons differed significantly between the sexes only in *A. fumiferanae* and *D. absona* (Table 2). The interaction between source of cocoons and sex was significant only for the length and ratio variables in *D. absona* (Table 2).

Webbing characteristics

The larval meconium is almost always visible as a dark, irregular mass at or near the base of the cocoon (Fig. 1). This must not be confused with dark banding caused by dense fibers of the cocoon. The apex of the cocoon is the cap end (*i.e.*, the end through which the adult parasitoid emerges).

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TABLE 3. Percentage of the cocoons of various species of the genera *Apanteles* and *Dolichogenidea* belonging to the types described in the text and illustrated in Figures 1–6.

Species	*n*	Percent type I	Percent type II	Percent type III
Apanteles fumiferanae	677	95.1	3.7	1.2
Apanteles petrovae	71	97.2	2.8	—
Apanteles morrisi	65	100	—	—
Apanteles milleri	3	100	—	—
Dolichogenidea absona	324	99.7	0.3	—
Dolichogenidea renaulti	5	100	—	—

Apanteles fumiferanae Viereck

Three types of cocoons were observed (Figs. 1*a*, 1*b*, 1*c*). Type I cocoons are translucent in the middle and usually at each end, with dense, dark bands, of variable length, on either side of the middle translucent band (Fig. 1*a*). This type is very common (Table 3). Type II cocoons are translucent at the base and apex (Fig. 1*b*) and are rare (Table 3). Type III cocoons are translucent only at the base (Fig. 1*c*) and are very rare (Table 3).

Apanteles petrovae Walley

Two types of cocoons were observed (Figs. 2*a*, 2*b*). Type I cocoons are translucent for about one third of the total length (Fig. 2*a*). This type is very common (Table 3). Type II cocoons are translucent for about half the total length (Fig. 2*b*) and are rare (Table 3).

Apanteles milleri Mason

Only one type (Fig. 3, Table 3) was observed. It is translucent only narrowly at the base, very similar to *A. fumiferanae* type III cocoons, but the darkness of the remainder of the cocoon is less pronounced.

Apanteles morrisi Mason

Only one type (Fig. 4, Table 3) was observed. It is not translucent anywhere along its length; dense fibers give these cocoons a uniform, whitish appearance.

Dolichogenidea absona (Muesebeck)

Two types of cocoons were observed (Figs. 5*a*, 5*b*). Type I cocoons are translucent at the base and apex, with a variable, dense, dark band of fibers in the middle (Fig. 5*a*), similar to *A. fumiferanae* type II cocoons, but with a much narrower band. This type is very common (Table 3). Type II cocoons are translucent for about the basal half (Fig. 5*b*), similar to *A. petrovae* type II cocoons, but in *A. petrovae* the translucent band is even greater than half the cocoon length. This type is very rare (Table 3).

Dolichogenidea renaulti Mason

Only one type (Fig. 6, Table 3) was observed. It is translucent except near the apex.

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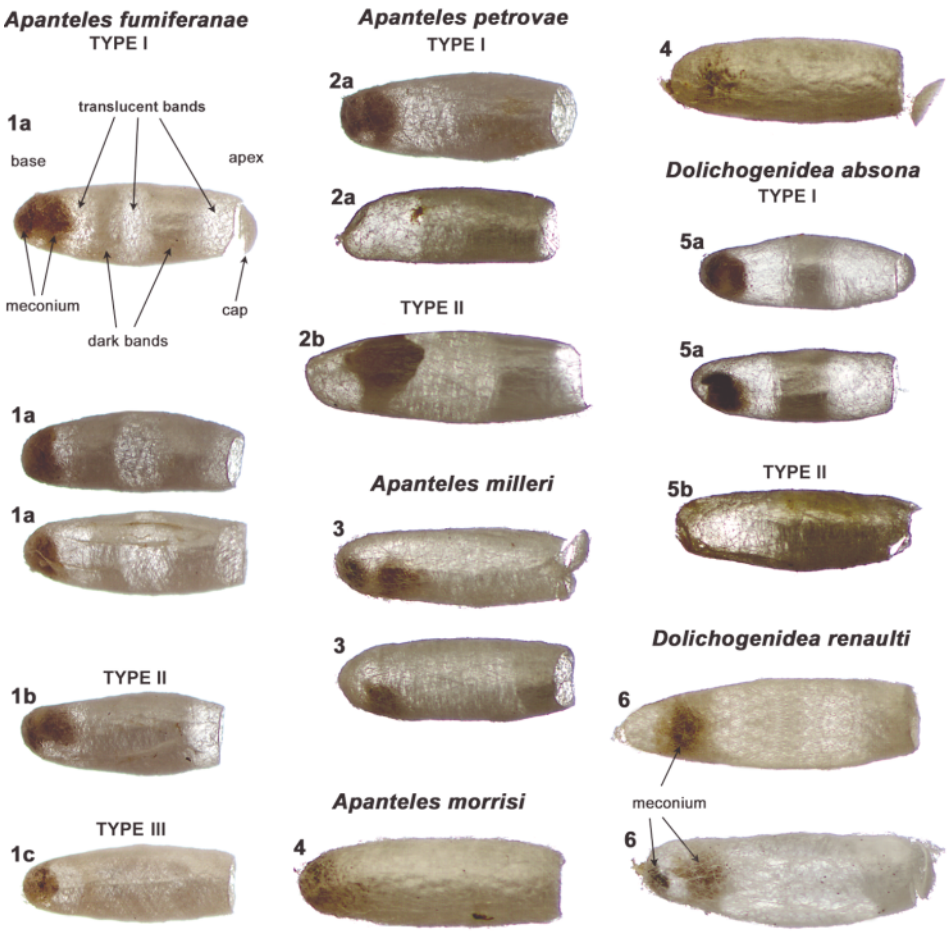

FIGURES 1–6. Cocoons of *Apanteles* and *Dolichogenidea* species obtained from *Choristoneura fumiferana* and other Microlepidoptera in the same microhabitat at the Acadia Research Forest, Fredericton, New Brunswick. Mean cocoon size of each parasitoid species is shown in Table 1.

Key to cocoons of the genera *Apanteles* and *Dolichogenidea* from *Choristoneura fumiferana* and related Microlepidoptera

1. Cocoon with dense fibers along entire length, giving it an opaque, uniform whitish appearance, except for brown meconium visible at the base (Fig. 4) . . . . . *Apanteles morrisi*
— Cocoon without dense fibers along entire length, thus with translucent bands contrasting with opaque, usually dark bands 2
2(1). Cocoon with 2 or 3 translucent bands, always with one at middle and at base, and often with one at apex as well, and with dark bands of dense fibers on each side of median translucent band (Fig. 1*a*) . . . . . *Apanteles fumiferanae* type I
— Cocoon with 1 or 2 translucent bands, but not translucent at middle 3
3(2). Cocoon translucent at both ends, with dark band of dense fibers in the middle (Figs. 1*b*, 5*a*) 4
— Cocoon translucent only at one end 5
4(3). Cocoon with dark median band about half entire length, longer than either translucent end (Fig. 1*b*) . . . . . *Apanteles fumiferanae* type II
— Cocoon with dark median band about one-quarter entire length, shorter than either translucent end (Fig. 5*a*) . . . . . *Dolichogenidea absona* type I

https://doi.org/10.4039/n03-023 Published online by Cambridge University Press

5(3). Cocoon narrowly translucent at base only, increasing in darkness towards apex (Figs. 3, 1*c*) . . . . . *Apanteles milleri* and *Apanteles fumiferanae* type III

— Cocoon more broadly translucent, at least one-third of length 6

6(5). Cocoon translucent for about one-third of its length (Fig. 2*a*) . . . . . *Apanteles petrovae* type I

— Cocoon translucent for about half its length or more 7

7(6). Cocoon translucent for about half its length (Figs. 5*b*, 2*b*) . . . . . *Dolichogenidea absona* type II and *Apanteles petrovae* type II

— Cocoon translucent for about basal three-quarters of its length (Fig. 6) . . . *Dolichogenidea renaulti*

Discussion

Because of considerable overlap in the range of length, width, and ratio of length to width of cocoons of both sexes of the six parasitoid species, and the effects of the source of cocoons on these variables, size measurements of cocoons cannot be used to identify individual species. The unique webbing pattern of each species, however, can be used to reliably identify their cocoons. *Apanteles morrisi*, *A. milleri*, and *D. renaulti* each appear to have unique webbing characteristics, whereas *A. fumiferanae*, *A. petrovae*, and *D. absona* each has at least one very common type that is unique. Thus, only in a few rare cases (<5%) will difficulties be encountered in distinguishing between species. Webbing characteristics of males and females of each species are similar, making it impossible to identify the sex of a species using any of the cocoon characteristics examined.

Although this study is based on the examination of empty cocoons, we found that the webbing characteristics of full, dead cocoons can be as easily determined as those of empty cocoons if the contents of the dead cocoons are carefully removed before examination. We conclude that this method of identifying species using webbing characteristics of the cocoons will be of value to researchers involved in estimating parasitism and hyperparasitism, and in identifying correct host–parasitoid associations.

Acknowledgements

We thank P McCarthy, G McDougall, and B Morin for assistance with collecting cocoons and rearing hosts. We thank B Mason and M Sharkey for identifying many of the adult parasitoids and K Bolte for photographing the cocoons and preparing the figures. We are grateful to P McCarthy, J Sweeney, G Thurston, and D Quiring for reviewing the manuscript.

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(Received: 26 March 2003; accepted: 11 June 2003)