

**NATURAL ENEMIES OF THE BEE GENUS *CERATINA*
(HYMENOPTERA: APIDAE) IN THE NIAGARA REGION,
ONTARIO, CANADA**

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Abstract

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Ceratina dupla and *C. calcarata* (Hymenoptera: Apidae) are abundant bees in southern Ontario, commonly nesting in staghorn sumac (*Rhus typhina*), wild raspberry (*Rubus strigosus*) and teasel (*Dipsacus fullonum*). *Ceratina* nests were collected from April-September 2008 and parasitized individuals were reared to adulthood in the laboratory. *Pyemotes* sp. (Pyemotidae) and *Baryscapus americana* (Eulophidae) were the most common natural enemies, followed by *Baryscapus* sp., *Axima zabriskiei*, and *Hoplocryptus zoesmairi*. *Eupelmus vesicularis*, *Coelopencyrtus* sp. and *Eurytoma* sp. near *apiculae* were rarely collected. This is the first record of *E. vesicularis* (Eupelmidae) as a primary host on any member of the family Apidae. New host records are also reported for *H. zoesmairi* (Ichneumonidae), two different *Baryscapus* spp. (Eulophidae), *Eurytoma* sp. near *apiculae* (Eurytomidae) and *Pyemotes* sp. (Pyemotidae) on *C. calcarata* and *C. dupla*. Detailed descriptions of immature development of the parasitoids, and their preferences for host bee species and host plant species are provided.

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Introduction

Parasite–host relationships have been studied for numerous species in a laboratory setting (Harri et al. 2008; Jervis et al. 2008; Traynor and Mayhew 2005). These studies are vital to help understand the dynamics of host–parasite interactions, however they often only involve the most common one or two parasitoids associated with the host under study. In a

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natural setting, hosts may be parasitized by a number of species at varying frequencies, each using different parasitism and developmental strategies at different times. By describing the life history, development and preferences of numerous parasite species attacking one host, a more complete understanding of these interactions is gained.

Bees of the genus *Ceratina* Latreille (often referred to as dwarf carpenter bees), are cosmopolitan, with the subgenus *Zadontomerus* Ashmead being found exclusively in the Western Hemisphere (Michener 2007). The life history of *Ceratina* offers an excellent opportunity to study the development and interactions of parasites with their hosts. All offspring from eggs laid by a single female can be collected together in a nest, thus allowing for observation of how the parasites interact with an individual host, as well as how nest substrate, position in the nest, and interactions with other parasites and the foundress bee occur.

The Niagara Region, Ontario, Canada, is home to two common species of *Ceratina* (*Zadontomerus*): *C. dupla* Say and *C. calcarata* Robertson. Their nests are commonly collected from staghorn sumac (*Rhus typhina* L.), wild raspberry (*Rubus strigosus* Michaux) and teasel (*Dipsacus fullonum* L.; J. Vickruck, unpub. data). Both sumac and wild raspberry are native to the region whereas teasel is an obsolete crop plant introduced from Europe, whose flower heads (when the seeds are mature) were once used to raise the knap on wool (Rector et al. 2006). Sumac and raspberry are both perennial plants found at wood margins, differing from teasel which is a biennial weed found in open, generally abandoned agricultural fields.

Ceratina in the Niagara region are solitary and univoltine, producing one brood per year and overwintering as newly emerged, unmated adults (J. Vickruck, unpublished data). Emergence and mating typically take place in mid-April, and new nests are founded in May. Nests are not reused from year to year and can only be initiated in twigs with exposed pith. After digging a linear tunnel females begin to forage, forming pollen and nectar provisions into rounded masses upon which a single egg is laid (Grothaus 1962; Johnson 1988; Kislow 1976). Each provision mass and egg is separated from its neighbours by a cell septum formed by the foundress. Once finished provisioning, females sit and guard the nest entrance until the eclosion of their offspring. The newly eclosed adults can either overwinter in their natal nest or disperse to found new hibernacula for the winter (Grothaus 1962; Kislow 1976).

Ceratina immatures can be classified into 18 developmental stages which were originally described by Daly (1966) for *C. dallatoreana*. The first eight stages rank the pupa in relation to the size of the pollen ball, after which the immature passes through a pre-pupal stage followed by metamorphosis. The eyes of the pupa then pass from white through to black (five stages), followed by darkening of the body (four stages). In the final stage the black bodied pupa emerges as an adult with milky wings.

Natural enemies of *Ceratina* in the Niagara region include predators, parasites, and parasitoids. Predators consume more than one prey individual in order to complete development. Parasites feed on the host contents but do not consume the entire host before completing development (Godfray 1994). Parasitoids consume a single host in order to complete development (Godfray 1994). Parasitoids were classified as idiobionts or koinobionts, endoparasitoids or ectoparasitoids, and gregarious or solitary. Idiobionts prevent the larva from developing further after initial parasitisation (Askew and Shaw 1986).

Koinobionts do not kill the host until it has reached a certain point in its development, as the parasitoid benefits from the continued life of the host (Askew and Shaw 1986). Ectoparasitoids develop outside the host (although they are often attached to it), while endoparasitoids consume the host internally. In solitary species the parasitoid to host ratio is 1:1, whereas in gregarious parasites multiple individuals develop in one host.

The objectives of this study were to identify and describe the development of the natural enemies of *Ceratina* in the Niagara Region as well as quantify their host and substrate preferences.

Materials and Methods

Host nest collections

All parasites were reared from a total of 107 nests of *C. calcarata* and *C. dupla* collected from 14 April to 30 September 2008. Each week at least 15 *Ceratina* nests were collected so that sampling effort was consistent over the season. Supplementary nest collections also took place in June 2009 to aid with final parasite identifications only. The 2009 data are not included in the statistical analysis. All collections took place at the Brock University campus (43.1197, -79.2492), the Glenridge Quarry Naturalization Site (43.1223, -79.2375) and an abandoned old field site near the Welland Canal (43.1479, -79.1811). Nests were collected from sumac, raspberry, and teasel and brought back to the laboratory in early morning to ensure that all occupants were present inside. After being chilled, twigs were carefully split open longitudinally to identify nest contents. Bee species, plant nest substrate, position of any parasitized cells in the nest, and developmental stages of bees and parasites were recorded on the day of collection. Dissected nests were then inserted in transparent PVC tubing slightly larger than the diameter of the nest (ranging from ½–1 inch depending on twig diameter) for protection and to allow for easy visual observation of nest contents. This also allowed for behavioural observations of host–parasite interactions in the laboratory.

Ceratina species were identified using the key of Rehan and Richards (2008). Parasite identifications were made by JTH and Dr. Gary Gibson at the Canadian National Collection of Insects, Arachnids and Nematodes (CNC), Dr. Michael Gates at the National Museum of Natural History (NMNH) in Washington, DC, as well as JV. Dr. Andrew Bennett (CNC) verified identity and nomenclature of *Hoplocryptus zoesmairi* Dalla Torre. Voucher specimens of *Baryscapus* sp. and *americana*, *Eupelmus vesicularis* (Retzius), *Coelopencyrtus* sp., *Axima zabriskiei* Howard and *Eurytoma* sp. near *apiculae*, were deposited in the CNC. *Baryscapus* sp., *Coelopencyrtus* sp., *Eupelmus vesicularis* and *Eurytoma* sp. near *apiculae* are labelled as CNC Ident. lot # 2008-341, and *Baryscapus* sp., *Axima zabriskiei* and *H. zoesmairi* as 2009-188.

Parasite development and classification

Hosts were observed on a daily basis to detect parasitoid presence. Position in the nest, stage parasitized, and parasitoid species were recorded as soon as they became apparent. Developmental milestones such as defecation, pupation, pigmentation of the exoskeleton as well as emergence dates were recorded for parasites whenever possible.

Once parasitoids had pupated they were transferred to their own individual 0.2 mL microcentrifuge tubes prior to eclosion. Upon emergence parasitoids were placed in 70% ethanol for later identification.

Data Analysis

All data were analyzed using SAS 9.1. Parasite prevalence is defined as the number of individuals affected by a particular parasite species divided by the number of hosts examined (Margolis et al. 1982). Parasite frequency is defined as whether that particular species is present in the nest, regardless how many individuals in the nest were parasitized. G-tests for goodness of fit were used when sample sizes were large, Fisher's exact tests were used when expected values were small (<5). *Hoplocryptus zoesmairi* has not been included in statistics as it is a predator.

Results

Host parasitism

Eight species of arthropod parasites representing two classes (Insecta, Arachnida), two orders (Hymenoptera and Trombidiformes), and seven families were reared from a total of 107 *C. dupla* and *C. calcarata* nests containing 850 brood cells. Characteristics of these eight species are compared in Table 1. Of the 107 nests collected, 64 were teasel, 36 raspberry, and 7 sumac. Twenty-nine percent (243/850) of all brood cells were parasitized, and 68% (73/107) of nests contained at least one parasite. *Ceratina calcarata* had a significantly higher proportion of cells parasitized than *C. dupla* but the proportion of nests parasitized between host species did not differ significantly (Table 2). Parasitism for each *Ceratina* species also varied by substrate, with significantly higher proportion of cells and nests parasitized in raspberry compared to teasel (Table 2). *Ceratina calcarata* was parasitized more often than *C. dupla* when nesting in raspberry ($G=20.05$, d.f.=1, $P<0.0001$), however no difference was seen between species in teasel ($G=0.04$, d.f.=1, n.s.) Sumac nests were not included in substrate comparisons due to small sample size. *Ceratina dupla* nesting in raspberry was the least parasitized with 15% of available cells affected (Table 2, Fig. 1). Only seven sumac nests were found, all *C. calcarata*, in which 16/33 (48%) of immatures had been parasitized (Fig. 1).

Parasite and predator development

The frequency and prevalence, i.e., proportion of hosts affected, of all parasites and predators in *Ceratina* nests is presented in Table 3 for affected cells and Table 4 for affected nests. Detailed observations for each species are given below.

Predators

Hoplocryptus zoesmairi (Dalla Torre) (Hymenoptera: Ichneumonidae)

This species has previously been associated with *C. dupla* (as *Habrocryptus graenicheri* Viereck; Viereck 1904). *Hoplocryptus* Thomson has until recently been considered a junior synonym of *Aritranis* (Förster) (Yu and Horstmann 1997), but Yu et

TABLE 1. Important developmental characteristics of natural enemies of *Ceratina dupla* and *C. calcarata* in the Niagara Region. The species are all Hymenoptera except *Pyemotes* sp. which belongs to Trombidiformes.

Parasite/Predator	Biology	Host Species	Host nesting substrate	Natural enemies per host	Host stages attacked	Previous host record
<i>Hoplocryptus zoesmairi</i> (Ichneumonidae)	Predator	<i>C. dupla</i> , <i>C. calcarata</i>	Teasel, Raspberry	Predator ^a	Larvae	Reported on <i>C. dupla</i> (Viereck 1904, Graenicher 1905), new host record <i>C. calcarata</i>
<i>Baryscapus americana</i> (Eulophidae)	Koinobiont Endoparasitoid	<i>C. dupla</i> , <i>C. calcarata</i>	Teasel, Raspberry	Gregarious (>10)	Prepupae, occasionally white eyed pupae	<i>B. americanus</i> reported from <i>C. calcarata</i> (Rau 1928, Kislow 1976), new host record <i>C. dupla</i>
<i>Baryscapus</i> sp. (Eulophidae)	Koinobiont Endoparasitoid	<i>C. calcarata</i>	Raspberry, Sumac	Gregarious (8-14)	Prepupae, white-eyed pupae ^b	See previous host records for <i>Baryscapus</i> sp. 1 above
<i>Coelopenycirtus</i> sp. (Encyrtidae)	Koinobiont Endoparasitoid	<i>C. calcarata</i>	Sumac	Gregarious (>20)	Medium larvae	<i>C. hylaei</i> reported on <i>C. calcarata</i> (Daly 1967)
<i>Eupelmus vesicularis</i> (Eupelmidae)	Idiobiont Ectoparasitoid	<i>C. dupla</i> ^c	Teasel	Solitary	White-eyed pupae	New host record for family Apidae as 1 ^o host
<i>Eurytoma</i> sp. near <i>apicalae</i> (Eurytomidae)	Idiobiont Ectoparasitoid	<i>C. calcarata</i>	Teasel	Solitary	Large larva	Reported on <i>Ceratina</i> (Bugbee 1966). New host record <i>C. calcarata</i>
<i>Axima zabriskiei</i> (Eurytomidae)	Idiobiont Ectoparasitoid	<i>C. dupla</i> , <i>C. calcarata</i>	Raspberry, Sumac	Solitary or Gregarious	Prepupae, white-eyed pupae	Reported on <i>C. dupla</i> and <i>C. calcarata</i> (Kislow 1976, Krombien 1960, Rau 1928)
<i>Pyemotes</i> sp. (Pyemotidae)	General Ectoparasite	<i>C. dupla</i> , <i>C. calcarata</i>	Teasel, Raspberry	Gregarious	All larval and pupal stages	New host record for both species

a) Multiple *Ceratina* immatures are consumed in the development of one larva.

b) Parasitoids overwinter as full grown larvae.

c) May be a hyperparasitoid on *Baryscapus americana*.

TABLE 2. Prevalence of natural enemies associated with *Ceratina dupla* and *C. calcarata* in each substrate. Due to low sample sizes sumac was excluded from statistical analysis of parasitism amongst substrates.

Species	Substrate	Prevalence (%)	
		Brood cells	Nests
<i>C. dupla</i>	Teasel	107/426	27/49
	Raspberry	15/97	9/10
	Total	122/523	36/59
<i>C. calcarata</i>	Teasel	25/96	8/15
	Raspberry	80/198	25/26
	Sumac	16/33	4/7
	Total	121/327	37/48
Grand Total		243/850	73/107
<i>C. dupla</i> vs. <i>C. calcarata</i>		G=18.42, d.f.=1, P<0.0001	G=3.21, d.f.=1, P=0.07
Teasel vs. Raspberry		G=4.44, d.f.=1, P=0.04	G=20.21, d.f.=1, P<0.0001

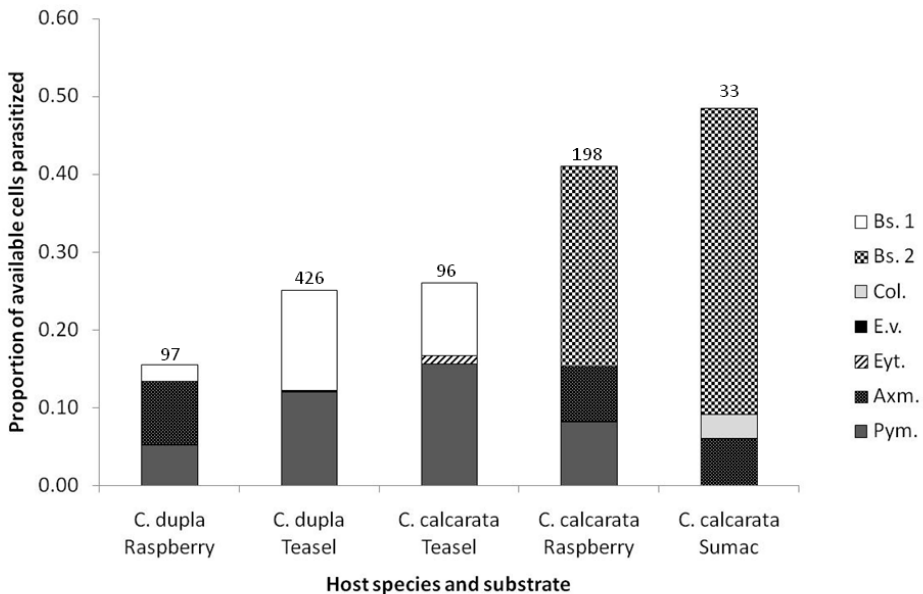


FIGURE 1. The proportion of available cells parasitized for *Ceratina dupla* and *C. calcarata* in each substrate. Values associated with each bar indicate the number of available cells for each species in each substrate. Abbreviations: Bs. 1.=*Baryscapus americana*, Bs. 2.=*Baryscapus* sp., Col.=*Coelopencyrtus* sp., E.v.=*Eupelmus vesicularis*, Eyt.=*Eurytoma* sp. near *apiculae*, Axm.=*Axima zabriskiei*, Pym.=*Pyemotes* sp.

TABLE 3. Prevalence of parasites on each *Ceratina* host by affected brood cells. Prevalence is the proportion of brood parasitized in each host species in each nesting substrate. Significance testing was conducted using G tests unless indicated by * where Fisher's Exact tests were used.

Parasite	Host	Substrate	Prevalence cells available	Statistics	
<i>Baryscapus americana</i> (Eulophidae)	<i>C. dupla</i>	Teasel	55/426	Species: G= 21.42, d.f.=1, P<0.0001	
		Raspberry	2/97		
		Total	57/523		
	<i>C. calcarata</i>	Teasel	9/96	Substrate: G=46.68 ,d.f.=1, P<0.0001	
		Raspberry	0/198		
		Sumac	0/33		
Total	9/327				
<i>Baryscapus</i> sp. (Eulophidae)	<i>C. calcarata</i>	Teasel	0/96	Substrate G= 2.49, d.f.=1, n.s.	
		Raspberry	51/198		
		Sumac	13/33		
		Total	64/327		
<i>Coelopenyrtus</i> sp. (Encyrtidae)	<i>C. calcarata</i>	Teasel	0/96		
		Raspberry	0/198		
		Sumac	1/33		
		Total	1/327		
<i>Eupelmus vesicularis</i> (Eupelmidae)	<i>C. dupla</i>	Teasel	1/426)		
		Raspberry	0/97		
		Total	1/523		
<i>Eurytoma</i> sp. near <i>apiculae</i> (Eurytomidae)	<i>C. calcarata</i>	Teasel	1/96		
		Raspberry	0/198		
		Sumac	0/33		
		Total	1/327		
<i>Axima zabriskieii</i> (Eurytomidae)	<i>C. dupla</i>	Teasel	0/426	Species: G= 8.02, d.f.=1, P=0.005	
		Raspberry	8/97		
		Total	8/523		
	<i>C. calcarata</i>	Teasel	0/96	Substrate: *X ² = 0.08, d.f.=1, n.s.	
		Raspberry	14/198		
		Sumac	2/33		
Total	16/327				
<i>Pyemotes</i> sp. (Pyemotidae)	<i>C. dupla</i>	Teasel	51/426	Species: G=0.53, d.f.=1, n.s.	
		Raspberry	5/97		
		Total	56/523		
	<i>C. calcarata</i>	Teasel	15/96		Substrate: G= 7.56 d.f.=1, P=0.006
		Raspberry	15/198		
		Sumac	0/33		
Total	30/327				

al. (2005) consider *Hoplocryptus* a valid genus. This is the first time this species has been recorded with *C. calcarata*.

This natural enemy is considered a predator as it always consumed multiple

TABLE 4. Infection rate of nests of *Ceratina* spp. by substrate type and species of natural enemy. Nests were scored as infected if at least one individual of the eight natural enemies were present in a nest. Significance testing was conducted using G tests unless indicated by * where Fisher's Exact tests were used.

Natural Enemy	Host	Substrate	Nests with at least one natural enemy/ Total nests	Statistics
<i>Baryscapus americana</i> (Eulophidae)	<i>C. dupla</i>	Teasel	13/49	Species: G= 10.16, d.f.=1, P=0.001
		Raspberry	2/10	
		Total	15/59	
	<i>C. calcarata</i>	Teasel	2/15	Substrate: G= 6.03, d.f.=1, P=0.01
		Raspberry	0/26	
Sumac		0/7		
Total	2/48			
<i>Baryscapus</i> sp. (Eulophidae)	<i>C. calcarata</i>	Teasel	0/15	Substrate: *X ² = 2.45, d.f.=1, n.s.
		Raspberry	16/26	
		Sumac	2/7	
		Total	18/48	
<i>Coelopencyrtus</i> sp. (Encyrtidae)	<i>C. calcarata</i>	Teasel	0/15	
		Raspberry	0/26	
		Sumac	1/7	
		Total	1/ 48	
<i>Eupelmus vesicularis</i> (Eupelmidae)	<i>C. dupla</i>	Teasel	1/49	
		Raspberry	0/10	
		Total	1/59	
<i>Eurytoma</i> sp. near <i>apiculae</i> (Eurytomidae)	<i>C. calcarata</i>	Teasel	1/15	
		Raspberry	0/26	
		Sumac	0/7	
		Total	1/48	
<i>Axima zabriskieii</i> (Eurytomidae)	<i>C. dupla</i>	Teasel	0/49	Species: G= 2.60, d.f.=1, n.s.
		Raspberry	4/10	
		Total	4/59	
	<i>C. calcarata</i>	Teasel	0/15	Substrate: *X ² = 0.87, d.f.=1, n.s.
		Raspberry	7/26	
		Sumac	1/7	
Total	8/48			
<i>Pyemotes</i> sp. (Pyemotidae)	<i>C. dupla</i>	Teasel	13/49	Species: G= 2.53, d.f.=1, n.s.
		Raspberry	3/10	
		Total	16/59	
	<i>C. calcarata</i>	Teasel	5/15	Substrate: G= 2.80, d.f.=1, n.s.
		Raspberry	2/26	
		Sumac	0/7	
Total	7/48			

Ceratina immatures before completing development. The predator egg was always laid in the innermost cell of the nest. After the egg hatched, the larva attached to the small *Ceratina* larva, but did not kill it immediately. Rather, the *H. zoesmairi* larva waited until the

Ceratina larva was at least half as large as its pollen mass, at which point it consumed the immature *Ceratina* and the remainder of its provisions. Once the entire contents of the cell had been consumed the larva broke down the cell septum and consumed the next larva and its pollen mass. This process was repeated, with individual *H. zoesmairi* larvae devouring anywhere from two to five *Ceratina* immatures and pollen masses, then spinning silken cocoons. Each *H. zoesmairi* larva then defecated and pupated inside its cocoon before emerging as an adult. Development from time of hatching to adulthood took 27-48 days, with emergence dates ranging from 28 July to 14 August 2008. There were four occurrences of this predator, two in *C. dupla* nests (one in teasel and one in raspberry), one in a *C. calcarata* nest (raspberry), and one in a *Ceratina* nest that contained no adult female and no surviving offspring.

Parasitoids

***Baryscapus americana* (Ashmead) (Hymenoptera: Eulophidae)**

Baryscapus americana was previously known to parasitize *C. calcarata* in Georgia (Kislow 1976) and Missouri (Rau 1928). The species was transferred from the genus *Aprostocetus* Westwood by Lasalle (1994). This is the first record of any member of the genus *Baryscapus* Förster parasitizing *C. dupla*.

Baryscapus americana is a gregarious, koinobiont endoparasitoid of *Ceratina* immatures. Their presence was undetectable until they began to consume their hosts (Fig. 2a), but the larvae grew to approximately half the length of their *Ceratina* host by the time its contents had been entirely consumed. At this point the parasitoids migrated to the anterior or posterior ends of the pre-pupal skin (Fig. 2b). The parasitoids then emerged in three ways: either all individuals in the *Ceratina* larval skin pupated and emerged that summer (Fig. 2c), or all of the individuals remained as prepupae to overwinter together and emerge the following spring, or several individuals occupying a single host would pupate while the rest would overwinter. The aforementioned strategies were also observed by Kislow (1976). Of the 66 immature *Ceratina* parasitized, 20 (30%) showed total emergence, 35 (53%) overwintered as a group together, and 11 (17%) showed partial emergence, with some individuals emerging that summer and some overwintering as prepupae. Average development time was 21.6 ± 2.3 days (range 11–37) once *B. americana* larvae had begun to consume *Ceratina* immatures. Emergence was highly synchronized for non-diapausing larvae, with all newly eclosed adults emerging from the host within 24 hours.

Baryscapus americana was the most common parasitoid species observed, infecting 8% (66/850) of all cells, and 16% (17/107) of all nests. This parasitoid was found in nests collected from 14 July through 1 August 2008. They were most often found parasitizing nests in teasel, with low levels of infection in raspberry, and none in sumac (Tables 3, 4). On average they infected 39% of available brood in an affected nest, ranging from one immature to the entire nest. This parasitoid predominantly affected the prepupal stage (8/9 *C. calcarata* and 53/56 *C. dupla*) and occasionally white eyed pupae. Individuals of *Baryscapus* sp. 1 were often found in nests with other associated species (7/17, 41%), including *Eurytoma* sp. near *apiculae*, *Axima zabriskiei*, *Eupelmus vesicularis* and *Pyemotes* sp.

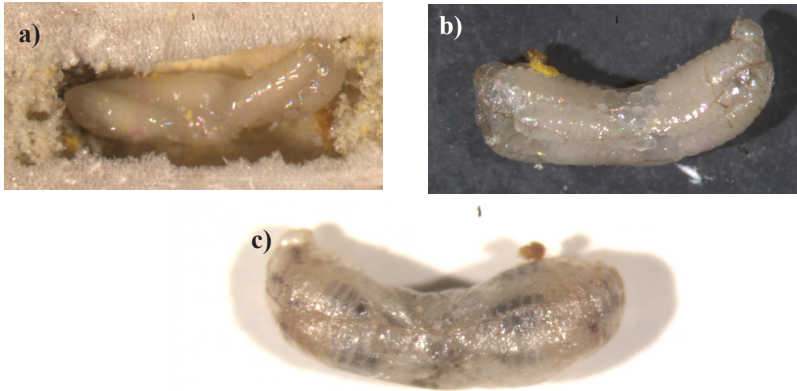


FIGURE 2. Development of *Baryscapus americana* a) Parasitoid larvae consume the contents of the *Ceratina* immature, leaving the larval skin intact. b) Full grown larvae move to the anterior and posterior ends of the host. c) Thereafter, pupation and development continue to eclosion or individuals overwinter as prepupae.

***Baryscapus* sp. (Eulophidae)**

This parasitoid which is morphologically very similar to *B. americana*, but mummifies its host, overwintered as prepupae in the larval or pupal skin of *Ceratina calcarata* only. All individuals of this gregarious, koinobiont endoparasitoid that emerged as adults were male. It infected 20% (64/327) of the total *C. calcarata* cells available and 17% (18/48) of all *C. calcarata* nests. It was found most commonly in raspberry (51 of 64 cells), occasionally in sumac (13 of 64 cells), and never in teasel. On average 3.8 ± 0.6 cells per affected nest were parasitized, representing 51% of infected *C. calcarata* nests on average. Other associated species were present in 8 of the 17 infected nests (47%); these were always *Pyemotes* or *Axima*. Prepupae were the most commonly affected host stage (43/64), but white-eyed pupae (21/64) were also susceptible to parasitism.

Parasitism went unnoticed until these internal parasitoids began to consume the host. Infection became evident when the larval skin of the *C. calcarata* changed dramatically in colour and consistency. The larval skin of living *Ceratina* is somewhat transparent and the gut is often visible. Parasitism caused the larval skin of the *Ceratina* to become a rusty red-brown colour; it also became much more brittle with the consistency of paper mache. The parasitoids overwintered as full grown larvae in the host, and the tough pupal casing of the larval or pupal skin may provide protection to the diapausing larvae (Legrand et al. 2004). Only males of this species emerged as adults from *Ceratina* immatures, in contrast with *Baryscapus americana* where both sexes emerged. These parasites were collected from *Ceratina* nests from 22 July through 21 August 2008.

***Coelopenecyrtus* sp. (Hymenoptera: Encyrtidae)**

A single *C. calcarata* larva in a sumac nest was affected by this gregarious,

endoparasitic koinobiont. The only other observation of *C. calcarata* being attacked by *Coelopencyrtus* Timberlake is by R.W. Matthews (reported by Daly et al. 1967), who reported *C. hylaei* Burks parasitism on six consecutive cells in a nest collected in Connecticut. *Coelopencyrtus* have also been reported to parasitize members of the twig-nesting, bee genus *Hylaeus* Fabricius (Burks 1958).

The *C. calcarata* nest was collected on 7 July 2008 and parasitism became evident on 10 July 2008 when more than 20 *Coelopencyrtus* larvae could be seen consuming the bee larva, which was in the second innermost cell in a nest with six other immatures. Once the entire contents of the *Ceratina* larva had been consumed, development of the parasitoids continued inside the transparent larval skin. Eyes of the parasitoids began to darken on 4 August with their exoskeletons gaining pigmentation by 7 August. Synchronized emergence took place on 13 August, when all of the new *Coelopencyrtus* adults emerged, except for one individual that had died during development.

***Eupelmus vesicularis* Retzius (Hymenoptera: Eupelmidae)**

One *Eupelmus vesicularis* specimen was reared from a *C. dupla* nest in teasel. While this is the first host record of *E. vesicularis* parasitizing *C. dupla*, members of the genus *Eupelmus* are well known for parasitizing a large number of different hosts (Burks 1979a; Gibson 1990). *Eupelmus vesicularis* has a Holarctic distribution, but may have been introduced to North America from Europe in straw (Burks 1979a). Its first record in North America was from Pennsylvania in 1915 (Burks 1979a).

Usually a primary parasitoid, *E. vesicularis* has been occasionally reported as a secondary parasitoid (Burks 1979a). The wasp collected here had actually parasitized a white-eyed bee pupa that had also been parasitized by *Baryscapus americana*. The *E. vesicularis* egg had already been laid when the nest was collected on 15 July 2008. The parasitoid hatched and began feeding externally on the bee larva on 20 July 2008. A day later it became apparent that the bee larva had also been parasitized internally by *B. americana*. *Eupelmus vesicularis* consumed the bee larva, followed by the *B. americana* parasitoids, and pupated on 1 August. Body sclerotization was quite rapid, beginning 4 August and finishing 2 days later. The adult *E. vesicularis* emerged on 8 August 2008, 19 days after first hatching. This is the first record of the family Eupelmidae associated with a parasitoid developing in bees.

***Eurytoma* sp. near *apicalae* Bugbee (Hymenoptera: Eurytomidae)**

This is the first record of *Eurytoma* Illiger parasitizing *C. calcarata*. *Eurytoma apicalae* and *E. nodularis* Boheman have been reported as parasitoids on *C. callosa* Fabricius, *C. dallatoreana* Friese, *C. nanua* Cockerell, and *C. punctigena* Cockerell (Bugbee 1966; Burks 1979b; Daly 1966), and an unknown *Eurytoma* species has been observed as a parasitoid of *C. australensis* Perkins in Queensland, Australia (S. Rehan, pers. comm.).

An external parasitoid of *C. calcarata*, only one *E. sp. near apicalae* individual was collected which was parasitizing a larva that had almost finished eating its pollen ball in a nest constructed in teasel. The *E. sp. near apicalae* egg was laid in the innermost brood cell and by 16 July, 2008, had begun to feed on the host *Ceratina* larva. Over the course of the next week the parasitoid finished consuming the host, after which it defecated and then

pupated. The eyes of the *E. sp.* near *apicalae* began to darken on 27 July and the integument was fully pigmented by 1 August. The teneral adult emerged on 3 August, 2008.

Axima zabriskiei Howard (Eurytomidae)

Axima zabriskiei has been reported as a parasitoid of both *C. dupla* and *C. calcarata* (Kislow 1976; Krombein 1960; Rau 1928). An ectoparasitic idiobiont, 1–7 *Axima* individuals could be seen consuming a single *Ceratina* immature, always a pre-pupa or white-eyed pupa, most often attached between the head and thorax and/or near the wing buds of white eyed pupae (Fig 3b). The parasitoids consumed the hosts' contents rapidly (usually in 24 – 48 hours), leaving the skin intact. It was at this point that most lab-reared parasitoids died, but two did pupate in the laboratory in 2008 (Fig. 3c). None of these chalcid parasitoids were successfully reared to adulthood in the lab in 2008 but one was reared to adulthood during 2009 collections.

Axima zabriskiei parasitoids infected 3% (24/850) of all available cells and 11% (12/107) of available nests. Twenty-two of the infected cells were found in raspberry (11 nests) and two cells were in sumac (one nest), for an average of 1.9 ± 0.3 cells per infected nest, with a maximum of four infected *Ceratina* immatures but never representing more than 50% of the total brood in a nest. *Axima zabriskiei* was found with other parasites

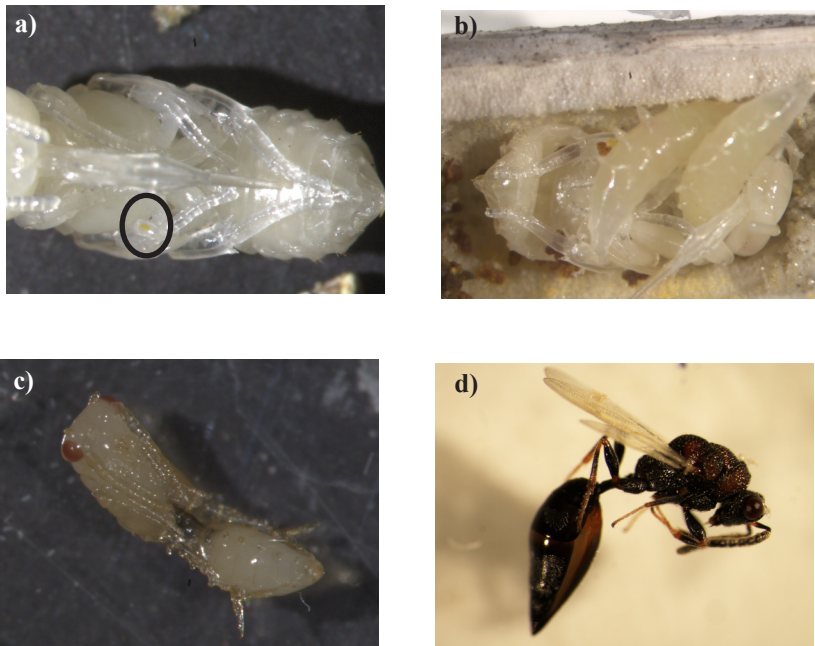


FIGURE 3. *Axima zabriskiei* wasp development. a) Newly hatched larvae pierce the soft exoskeleton of the pupa and rapidly ingest the contents (a, circle), usually within 24–48 hours. Often multiple parasitoids will attack a single *Ceratina* immature (b). Once finished feeding larvae pupate (c) before emerging as an adults (d).

in 7/12 (58%) affected nests, most often in conjunction with *Baryscapus* sp.. The first *Ceratina* nest containing *Axima zabriskiei* was collected on 14 July, with the last parasitized nest collected on 21 August 2008.

Parasites (other than parasitoids)

***Pyemotes* sp. (Acari: Actinedida: Pyemotidae)**

Pyemotes sp. were the most common natural enemy found on *Ceratina* immatures, infecting 10% (86/850) of all available brood cells and 21% (23/107) of all available nests. This is the first record of *Pyemotes* mites infecting *C. dupla* and *C. calcarata*, although they have been reported on *C. dallatorreana* (Friese) in California (Daly 1966). They were more common in teasel nests (66 of 86 infected brood) than in raspberry (20 of 86 infected brood), and were not found in sumac (Table 3). On average *Pyemotes* sp. affected 3.7 ± 0.7 immatures per nest, representing 28% of the total brood in affected nests. *Pyemotes* sp. was present in nest collections from 25 June to 25 July 2008.

This external parasite was found to infect all immature stages, from small larvae to fully pigmented pupae. Multiple individuals often infected a single larva or pupa, but a single mite was effective in paralyzing and killing the host. *Pyemotes* seemed to monopolize parasitism in a nest, being found with other parasitoids only 22% of the time (5/23 nests). *Pyemotes* mites were also observed feeding on two *A. zabriskiei* larvae which subsequently died. Other members of the genus *Pyemotes* have been known to decimate nests of the bee *Melipona colimana* Ayala and the stem-nesting wasp, *Psenulus interstitialis* Cameron (Macias-Macias and Otero-Colina 2004; Matthews 2000).

Discussion

Many parasitoids were more prevalent in one substrate than in another. *Baryscapus americana* for example, was collected significantly more often from teasel nests, with only two cells parasitized in raspberry (Table 3). While not statistically significant, *Axima zabriskiei* was collected more in raspberry than sumac (Table 3). *Pyemotes* mites did not parasitize one species more than another, but were significantly more common in teasel nests than they were in raspberry (Tables 3 and 4). While *Baryscapus* sp. was found parasitizing 64 individuals in 18 nests, it was only ever a parasite of *C. calcarata* in raspberry and sumac, never in teasel.

The parasitoid preferences seen for specific host substrates may be due to a number of factors such as the structure or biology of the host plant itself. The following discussion pertains mainly to raspberry and teasel, because so few sumac nests were collected. One possible reason for higher parasitism rates in raspberry than in teasel may be the structure of the plant species used for *Ceratina* nests. Teasel nests can only contain one nest per plant, in the straight stalk that grows perpendicular to the ground. Shrubs like raspberry (and sumac) have multiple branches in each plant and thus multiple possible nest substrates. These shrubs also tend to grow in aggregations, with multiple plants in very close proximity to one another. This can lead to higher nest densities in raspberry than in teasel. As *Ceratina dupla* and *C. calcarata* females guard only their own nests, the high density of nests in shrubs may

lead to increased rates of parasitism, as an individual parasitoid may be able to efficiently locate and infect several nests in close proximity. When comparing parasitism rates for a number of non-social hymenopteran species that nest solitarily and in aggregations, Rosenheim (1990) found that aggregated nests had higher parasitism rates in most cases.

Higher parasitism rates in raspberry may also relate to the habitat and biology of the plants in which *Ceratina* nest. Teasel is an invasive plant found in large open fields, almost always in full sunlight, while raspberry and sumac are both native plants located in shaded wood margins. In other words, *Ceratina* are nesting in different microclimates, in substrates with different biology, and with different possible chemical signatures. Numerous experiments have shown that many parasitoids are attracted to chemical cues of the flora where their host species are commonly found (Drost et al. 1986; Elzen et al. 1986; Godfray 1994; Vet 1983). If parasitoids use the microclimate and/or chemical cues emitted by the native substrates, then this might explain why the nests in native shrubs had higher parasitisation. Members of the genus *Eupelmus* parasitize a very wide range of host species (Gibson 1990). Gibson (1990) hypothesized that *Eupelmus* searched for hosts in specific microclimates, with the microclimate being of more importance than the host species. Searching for hosts by their preferred substrate may also be more effective in temperate regions due to the relatively short and synchronized phenology of foraging insects and nest substrates (Wcislo 1987).

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