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September 2011

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This article was originally published at:

<http://dx.doi.org/10.1155/2011/910286>

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Citation for this paper:

Lucarotti, C.J., Whittome-Waygood, B.H. & Levin, D.B. (2012). Histology of the Larval *Neodiprion abietis* (Hymenoptera: Diprionidae) Digestive Tract. *Psyche*, 2011, 10 pages. <http://dx.doi.org/10.1155/2011/910286>

## Research Article

# Histology of the Larval *Neodiprion abietis* (Hymenoptera: Diprionidae) Digestive Tract

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Received 18 April 2011; Revised 17 August 2011; Accepted 6 September 2011

Academic Editor: Robert Matthews

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The alimentary canal of *Neodiprion abietis* larvae is a straight tube divided into foregut, midgut, and hindgut. Posterior to the mouth, the foregut is further divided into the pharynx, esophagus (crop), and proventriculus, all of which are lined with cuticle. A pair of muscular, chitin-lined pouches branch off the anterior foregut and lie lateral to the alimentary canal. Gastric caeca are located at the anterior end of the midgut, where the peritrophic membrane is formed and was observed throughout the midgut. A single layer of midgut columnar epithelial cells abuts on the basal lamina at one end with microvilli extending into the gut lumen at the other. Nidi of regenerative cells were observed between columnar epithelial cells at the basal lamina. Malpighian tubules are attached to the posterior end of the midgut. The hindgut consists of the pylorus, a muscular ileum connecting to a bulbous rectum, which then opens to the anus.

## 1. Introduction

Insect gut morphology and function are dependent on several factors including insect taxon, developmental stage, feeding behavior, and food source [1, 2], but all insect guts follow the same basic plan. The fore- and hindguts originate from the embryonic ectoderm and are lined with cuticle [2–4]. The middle component, or midgut, has no cuticular covering and is generally thought to originate from the embryonic endoderm [2, 5, 6]. The foregut typically functions for short-term food storage and transport to the midgut [3], where food is digested by enzymes and nutrients are absorbed across a columnar epithelium. The hindgut is divided into the pylorus, ileum, and rectum, where water and salts may be absorbed, and the anus through which feces pass from the body [2, 4]. Malpighian tubules attach at the junction of the midgut and hindgut and may be on one side or the other of this junction depending on the insect [7, 8]. In 1895, Bordas [9] described the gut morphologies of selected

examples from every family in the Hymenoptera. Sixty years later, Maxwell [10] made extensive comparisons of the internal larval anatomies of 132 different species from 11 families of sawflies, collected worldwide, in an effort to resolve certain issues related to the taxonomy of sawflies. Maxwell [10] determined that the two major internal anatomical features for establishing evolutionary relationships amongst and between sawfly taxa were the salivary glands and Malpighian tubules.

The balsam fir sawfly, (*Neodiprion abietis*) (Hymenoptera: Diprionidae), is indigenous and widespread in North America, where the larvae feed predominantly on balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), and black spruce (*Picea mariana*) [11]. *Neodiprion abietis* is likely a species complex where temporal differences in life histories and host-plant selection for oviposition and feeding may provide an isolating mechanism for strains that can otherwise freely interbreed [12]. On the island of Newfoundland (Province of Newfoundland and Labrador (NL), Canada),

outbreak populations of balsam fir sawflies typically occur in 5- to 15-year cycles and last 4 to 5 years [13]. Here, balsam fir sawfly larvae emerge in late spring and early summer after overwintering as eggs that had been oviposited the year before in the then current-year needles of balsam fir trees. Male larvae pupate after the fifth larval stadium, whereas female larvae may go through an additional instar before pupating [14]. The adults emerge in late summer, and mated females will lay female eggs and unmated females, male eggs (arrhenotoky). Outbreak populations of balsam fir sawflies are brought down by epizootics of a naturally occurring nucleopolyhedrovirus (NeabNPV: *Gammabaculovirus: Baculoviridae* [15]) [16]. Sawfly NPVs only infect the midgut [17], so prior to examining the pathology of NeabNPV in larval balsam fir sawflies and because of the general paucity of reports on sawfly gut anatomy, we have first undertaken an examination of the anatomy and histology of the healthy alimentary canal of the balsam fir sawfly larva.

## 2. Materials and Methods

**2.1. Larval Collection.** Balsam fir branches, containing *N. abietis* larvae, were collected from the leading edge of the balsam fir sawfly outbreak near Old Man's Pond, NL, Canada (49°7'23.3''N: 57°51'45.6''W) between 18–21 July 2003. Larvae were maintained on balsam fir foliage in 20-kg brown paper bags at 4°C for a maximum of 48 h. Larvae were removed from the foliage, and head capsule measurements were taken using a dissecting microscope equipped with a calibrated micrometer in the objective lens. Larvae with head capsule widths between 0.96 to 1.5 mm, which correspond to third- to fifth-instar larvae [14], were transferred to sterile 100 mm × 15 mm plastic Petri dishes for a 12- to 15-h starvation period. Larvae were then allowed to imbibe an aqueous solution of 10% pasteurized honey and were placed on clean (5-min soak in 0.25% aqueous NaOCl followed by three 15-min rinses in tap water), fresh balsam fir sprigs, for 72 h at ambient room temperature (approximately 20°C) to monitor for signs of NeabNPV infection.

### 2.2. Histological Preparations

**2.2.1. Paraplast Sections.** Larvae were submerged whole in Bouin's fixative (Electron Microscope Sciences, Hatfield, Pa, USA) for 24 to 48 h. Larvae were rinsed and dehydrated in a graded ethanol series to butanol and embedded in Paraplast+ (Sherwood Medical, St. Louis, Mo, USA). Embedded material was sectioned using a steel histological knife on an American Optical (Buffalo, NY) 820 Spencer rotary microtome set to cut sections 10 µm thick. Serial sections on clean glass microscope slides were stained using a modified azan staining technique [18], dehydrated in ethanol to Hemo-D (Fisher Scientific, Fair Lawn, NJ, USA) and mounted in Permount (Fisher Scientific, Fair Lawn, NJ, USA).

**2.2.2. Epon-Araldite Sections.** Larvae harvested for whole-mount preparations and epoxy embedding were first injected

with fixative (2.5% glutaraldehyde—0.05 M sodium cacodylate—0.1 M sucrose pH 7.4) using a 1-cc, 27G1/2 syringe and needle (Becton Dickinson, Franklin Lakes, NJ, USA). The heads and tails were removed at the head capsule and eighth proleg, respectively, and then, the gut was pulled from the hemocoel into fresh fixative, using fine forceps. Guts excised for epoxy embedding were then embedded in 2% low-gelling-temperature agarose (SeaPlaque: FMC Bio-Products, Rockland, Me, USA) to preserve the integrity of the gut contents [19]. Guts were then cut roughly into fore-, mid-, and hindgut sections, re-embedded in agarose, and transferred to 1.5-mL microcentrifuge tubes containing fresh fixative overnight at 4°C. Gut samples were rinsed at 20°C for 15 min each in 0.05 M sodium cacodylate containing 0.1 M sucrose, 0.05 M sucrose and no sucrose followed by postfixation in 1% OsO<sub>4</sub> in 0.05 M sodium cacodylate buffer, pH 7.4 for 1 h at 20°C. Samples received two 15-min rinses in the same buffer followed by two 10-min washes in distilled water prior to en bloc staining in 4% aqueous uranyl acetate overnight and in the dark at 4°C [19]. Two additional 10-min water washes were followed by dehydration in a graded acetone series (30–100%) and embedding in Epon-Araldite [20] (Epon-5 g, Araldite-5 g, DDSA-12 g, DMP-30–500 µL) (Electron Microscope Sciences, Hatfield, Pa, USA). Sections were cut using an RMC MT-7 ultramicrotome at 1 µm for light microscopy and 80–90 nm for electron microscopy using Diatome (Biel, Switzerland) Histo and diamond knives, respectively. Thin sections were placed on clean glass slides, stained with toluidine blue-basic fuchsin (Canemco-Marivac, St. Laurent, QC, Canada) and were mounted in Permount. Ultrathin sections on 100-mesh copper grids were stained with 2% uranyl acetate and 0.01% lead citrate. Digital light microscope images were captured using a Nikon Eclipse 80i/DS camera (Nikon Instruments, Melville, NY, USA). Electron microscope images were taken on an Hitachi H7000 transmission electron microscope (Mississauga, ON, Canada) at 75 kV.

## 3. Results

**3.1. The Alimentary Canal.** The *N. abietis* larval alimentary canal is straight and runs the full length of the body of the larva, between the anterior mouth and posterior anus, and is divided into three distinct regions (Figures 1 and 2): foregut (Figures 3–9), midgut (Figures 9–13), and hindgut (Figures 13–19). The foregut forms approximately 22% of the total length of the gut. A pair of muscular diverticular pouches branch off the anterior foregut (Figures 1, 3, and 4) and connect to the buccal cavity (Figure 5). Like all components of the foregut, these pouches are lined with cuticle. Posterior to the buccal cavity is the muscular pharynx with posteriorly directed cuticular spines (Figures 6–8) followed by the esophagus that enlarges posteriorly to form the crop (Figures 6 and 9). The proventriculus (Figures 9 and 10) is characterized by a thick cuticle and is the last part of the foregut before the midgut. Gastric caeca encircle the anterior end of the midgut, and it is in this region that peritrophic membrane is formed by cells of the cardia and anterior

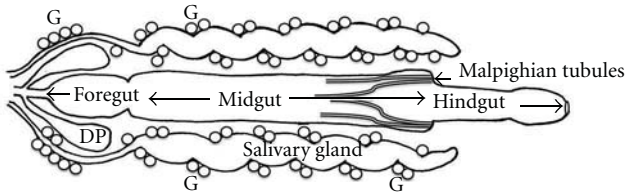


FIGURE 1: Diagrammatic representation of larval *Neodiprion abietis* salivary glands, gland cells (G), diverticular pouches (DP), and alimentary canal (after Maxwell [10]).



FIGURE 2: Photomontage of a whole mount of the foregut (FG), midgut (MG), and hindgut (HG) of a balsam fir sawfly larva. The arrow on the left indicates the junction of the foregut and midgut and the arrow on the right indicates the junction of the midgut and hindgut, where the Malpighian tubules (to the right of the arrow) are inserted. Scale bar = 2 mm.

midgut (Figures 9 and 10). The midgut is about 63% of the length of the alimentary canal, the majority of which consists of columnar epithelial cells butting onto the basal lamina which is then surrounded by circular and longitudinal muscles (Figures 11 and 12). Nidi of regenerative cells lie between columnar epithelial cells and adjacent to the basal lamina (Figures 11). At the posterior end of the midgut, just in front of the pylorus of the hindgut, the epithelium forms folds (Figures 13 and 14), and the cells in this region appear to be contributing material to the peritrophic membrane (Figure 15). Malpighian tubules were observed to insert into the posterior end of the midgut just anterior to the pylorus (Figure 14). The hindgut makes up the remaining 15% of the alimentary canal, consisting of the pylorus, ileum, rectum, and anus (Figures 13–19), all of which are lined with cuticle. The pylorus is not as wide as the midgut and constricts further at the ileum (Figures 2 and 13), which forms a muscular tube characterized by a thick cuticle (Figures 16 and 17). The epithelium undulates and consists of cuboid cells (Figure 17). Posterior to the ileum is the bulbous rectum, where waste plant material could be seen encased in a sheath formed by the peritrophic membrane (Figure 18). The outer surface of the cuticle lining of the anus had posteriorly directed spines and innervated setae (Figure 19).

**3.2. The Salivary Glands.** A pair of salivary glands flank the alimentary canal and open into the buccal cavity (Figure 1). The glands consisted of pair of large ducts, each with numerous gland cells on their surfaces (Figures 20–25). Granules were observed within and outside the cytoplasm of the gland cells (Figures 22 and 23). The salivary ducts consisted of a single layer of epithelial cells with microvilli facing the central lumen of the ducts, where secretions were observed (Figures 20, 24, and 25).

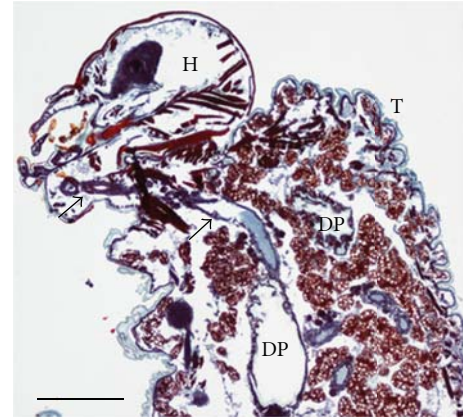


FIGURE 3: Longitudinal section through the head (H) and thorax (T) of a *N. abietis* larva showing the paired diverticular pouches (DP) and the tubule (arrows) that connects one of the pouches to the buccal cavity. (Note that serial sections showed the continuity of the tubule between the diverticular pouch and the buccal cavity.) Scale bar = 0.5 mm.

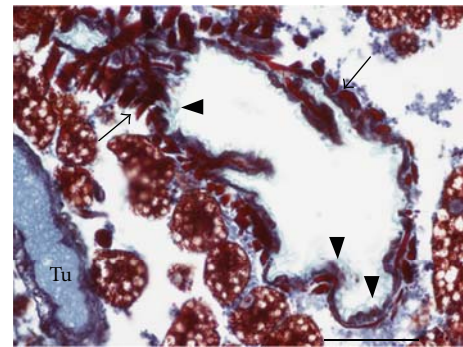


FIGURE 4: Detail of the musculature (arrows) and cuticle lining (arrowheads) of a diverticular pouch (different section but same pouch as the one on right in Figure 3) and tubule (Tu) with contents of a second pouch (on left in Figure 3). Scale bar = 0.1 mm.

Figures 3–6, 9, 10, 13–16, and 18–21 are light micrographs of paraplast sections. Figures 7, 8, 11, 17, and 22–25 are light micrographs of epoxy sections, and Figure 12 is an electron micrograph of an epoxy section.

#### 4. Discussion

Sawflies are phytophagous and the Diprionidae are defoliators of conifer trees (Pinaceae) with digestive systems adapted to that purpose. The diverticular pouches, which branch off the diprionid larval foregut, are used to store host-plant-derived terpenoids that are regurgitated in defensive behaviors against predators [21–27]. Like *N. abietis*, the diverticular pouches of *N. sertifer* larvae have been shown to be muscular sacs lined with an impervious layer of cuticle [21]. The chemistry of the contents of the pouches of conifer-feeding diprionid sawflies [21–24] and pergid sawflies feeding on eucalyptus [28] reflect the chemistry of the food source. Food source and larval stadium can also

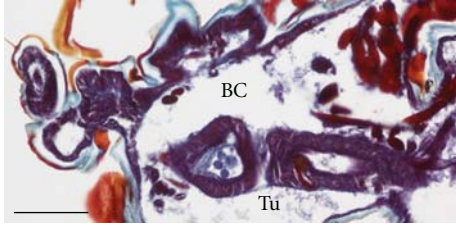


FIGURE 5: Detail of tubule (Tu) extending into the buccal cavity (BC) in the head of a *N. abietis* larva. (Same larva, different section as Figure 3.) Scale bar = 0.1 mm.

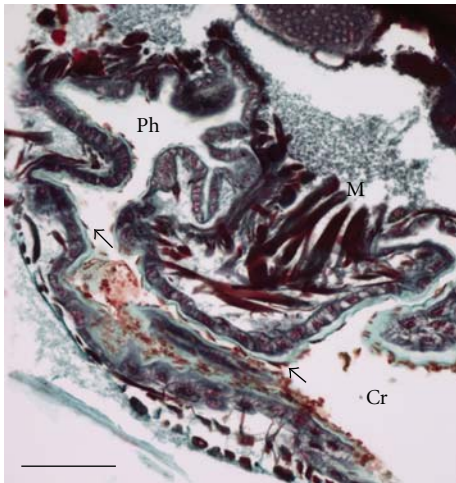


FIGURE 6: Muscular (M) pharynx (Ph) and anterior crop (Cr) of a *N. abietis* larva. Cuticular spines of the pharynx (arrows) are directed posteriorly. Scale bar = 0.1 mm.

affect the volume of fluid regurgitated. Redheaded pine sawfly (*N. lecontei*) larvae fed *Pinus banksiana* regurgitated  $0.26 \pm 0.02 \mu\text{L}$  compared with  $0.07 \pm 0.04 \mu\text{L}$  when fed *P. resinosa* [24]; second-instar red pine sawflies (*N. nanulus nanulus*) released  $58.9 \pm 2.3\%$  of the regurgitated volume on the first expulsion of fluid and lower amounts with each of the next two expulsions, whereas fifth instars released  $40.5 \pm 2.0\%$  on the first regurgitation and lower but similar amounts on the next two regurgitations [24]. *Diprion pini* larvae fed a high-resin diet produced higher amounts of fluid and had higher pupal weights than larvae fed low-resin diets, indicating that the cost and maintenance of this chemical defense was low [27]. However, there may be a balance between negative effects of high-resin acid contents to early instars (e.g., longer development times) and advantages of positive effects (chemical defense) to late instars in *N. sertifer* larvae [23]. Balsam fir sawfly larvae have been shown to perform best (better survival and cocoon weights) when they could feed on all age classes of foliage [29].

The rest of the foregut is involved in the intake of food, its trituration, and movement back towards the midgut [2, 3]. The musculature and posteriorly directed spines on the cuticle of the pharynx of *N. abietis* larvae would aid in these functions. The esophagus is enlarged to serve as a temporary

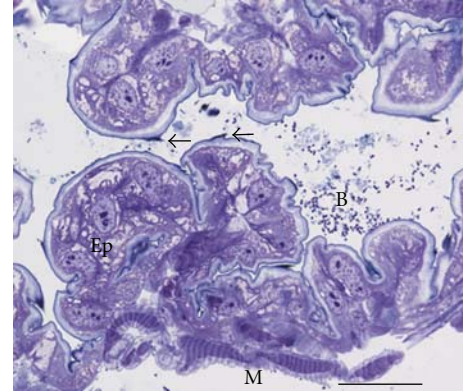


FIGURE 7: Posteriorly directed cuticular spines (arrows) subtended by a single cell layer of epithelium (Ep) and muscle (M). Bacteria (B) can be seen in the lumen of the pharynx. Scale bar =  $30 \mu\text{m}$ .

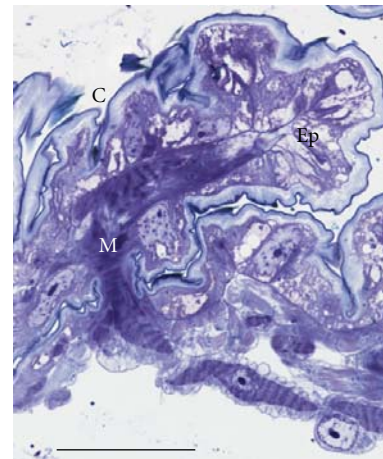


FIGURE 8: Detail of the epithelium (Ep), muscles (M), and cuticle (C) of the pharynx of a *N. abietis* larva. Scale bar =  $30 \mu\text{m}$ .

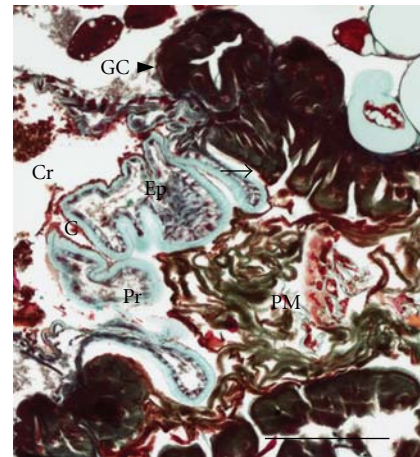


FIGURE 9: Posterior end of the foregut (left) showing the crop (Cr) and proventriculus (Pr) with its thick cuticle (C) and subtending epithelium (Ep) and the anterior end of the midgut (right) with a gastric caecum (GC) and peritrophic membrane (PM) being produced by cells of the cardia (arrows). Scale bar = 0.2 mm.

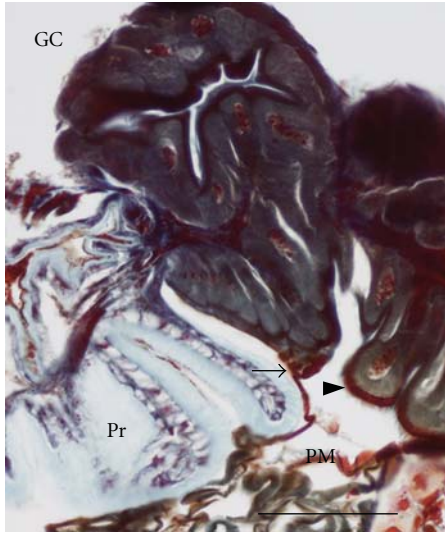


FIGURE 10: Junction of the foregut (left) and midgut (right) showing the proventriculus (Pr), a gastric caecum (GC), and the origin (arrow) of the peritrophic membrane (PM). The anterior cells of the midgut also appear to contribute secreted material (arrowhead) to the peritrophic membrane. Scale bar = 0.1 mm.

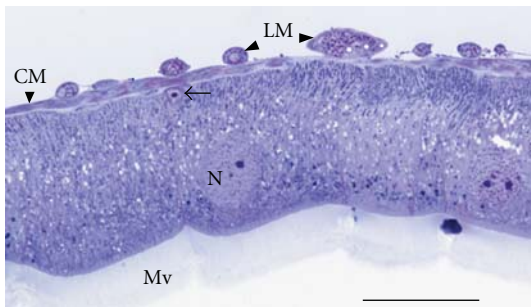


FIGURE 11: Nucleated (N) columnar epithelial cells of the midgut with microvilli (Mv) extending into the lumen (endoperitrophic space) of the gut. A regenerative cell (arrow) lies between two columnar epithelial cells beneath layers of circular (CM) and longitudinal (LM) muscle. Scale bar = 30  $\mu\text{m}$ .

storage organ, and the proventriculus, with its thick cuticle covering, is involved in the maceration of food [2, 3]. The cardia are specialized cells of the proventriculus that secrete the peritrophic membrane [30]. Peritrophic membranes laid down by cardia cells only are considered type II peritrophic membranes and are found, for example, in larval Diptera and a few adult Lepidoptera [31]. Type I peritrophic membranes are produced along the entire length or at either end of the midgut [1]. Type I peritrophic membranes are found in Coleoptera (beetles), Dictyoptera (cockroaches), Hymenoptera (bees, ants, wasps), Lepidoptera (moths and butterflies), and adult hematophagous Diptera (e.g., female mosquitoes) [31]. In larval *N. abietis*, material used in the formation of the peritrophic membrane was observed being secreted by the cells of the cardia (proventriculus) and anterior and posterior midgut epithelium. The evolution of the cardia may be an adaptation allowing insects to produce large

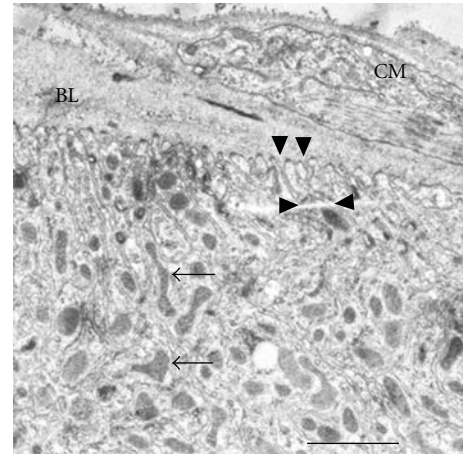


FIGURE 12: The base of a midgut columnar epithelial abuts against the basal lamina (BL) and circular muscle (CM). Fine, finger-like projections of the basal lamina cause invaginations of the epithelial cell plasma membrane (arrowheads). Mitochondria in the epithelial cell are indicated by the arrows. Scale bar = 1  $\mu\text{m}$ .

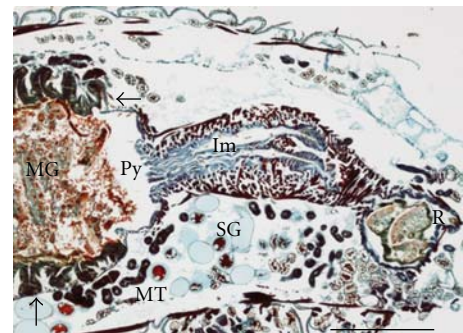


FIGURE 13: Longitudinal section (dorsal side up) of a *N. abietis* larva showing folds (arrows) in the epithelium at posterior end of the midgut (MG) and the hindgut showing the pylorus (Py), ileum (Im), and rectum (R). Malpighian tubules (MT) and salivary gland (SG) are visible in the hemocoel. Scale bar = 0.5 mm.

quantities of peritrophic membranes at a localized region of the gut [32]. In larval sawflies, the importance of abundant production of peritrophic membrane in the anterior region of the midgut would be for the protection of the midgut epithelium from abrasion by the jagged edges of the partially masticated food [31] and potentially harmful microbial pathogens while allowing for the passage of molecules (enzymes and products of digestion) between the endo- and ectoperitrophic spaces [30]. In Hymenoptera, initial stages of digestion occur in the endoperitrophic space, intermediate stages in the ectoperitrophic space, and final stages at the surface of the midgut epithelium [31]. Only the enzymes involved in the initial stages of digestion are free to move across the peritrophic membrane between the endo- and ectoperitrophic spaces [31]. A counterflow of water from the posterior midgut to the caeca is thought to recirculate these enzymes, in the ectoperitrophic space, to the anterior midgut, where they can re-enter the endoperitrophic space [31].

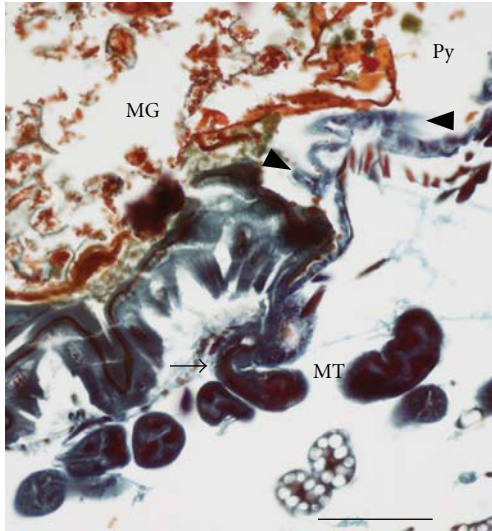


FIGURE 14: Insertion point (arrow) of a Malpighian tubule (MT) at the posterior end of the midgut (MG) just anterior of the pylorus (Py). Note the layer of cuticle (arrowheads) lining the pylorus, which is typical of the hindgut. Scale bar = 0.1 mm.

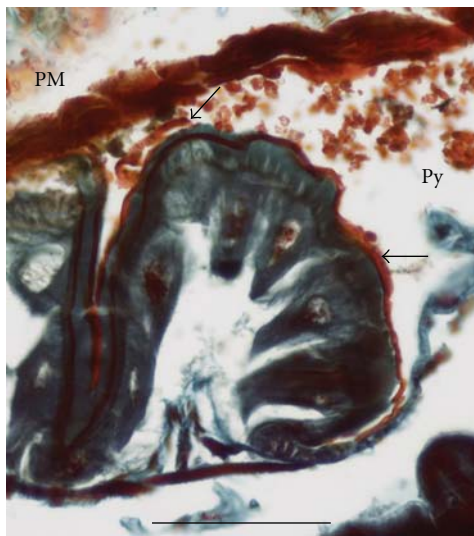


FIGURE 15: Cells contributing material (arrows) to the peritrophic membranes (PM) at the posterior end of the midgut just anterior of the pylorus (Py). Scale bar = 0.1 mm.

Sawfly larvae have distinct gastric caeca [10]; however, there is an evolutionary trend in the Hymenoptera, towards the Apocrita, where gastric caeca are lost and their function replaced by cells in the anterior midgut [31]. At the other end of the *N. abietis* larval midgut, Malpighian tubules empty into the ectoperitrophic space just anterior to the pylorus of the hindgut. Maxwell [10] reports that there are approximately 28 Malpighian tubules in *N. swainei* larvae and as she states that, “The general anatomy of all species of *Neodiprion* is monotonously similar; differences found on a minor level are described for *swainei*, *abietis*, and *lecontei*”, and one may assume a similar number are present in *N. abietis*. In her

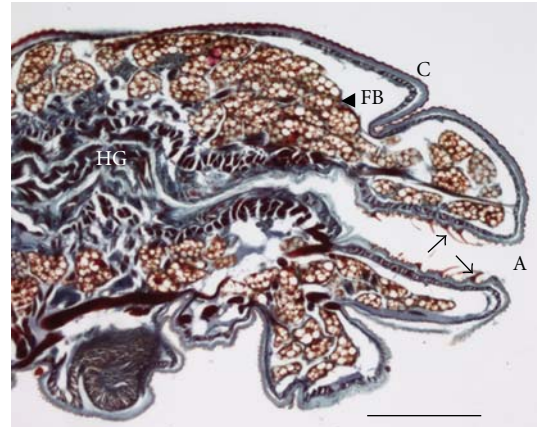


FIGURE 16: Longitudinal section (dorsal side up) of the posterior end of a *N. abietis* larva showing the muscular and chitin-lined hindgut (HG), which opens to the exterior through the anus (A). Anal setae are indicated by the arrows. Also visible are fat body (FB) and the exterior cuticle (C) of the larva. Scale bar = 0.2 mm.

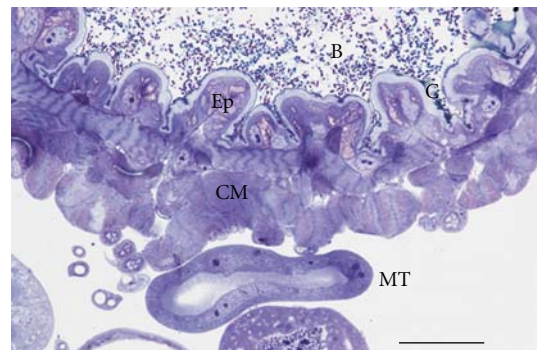


FIGURE 17: Detail of the ileum showing bacteria (B) in the gut lumen, the thick cuticle (C), single layer of epithelial cells (Ep), and layers of circular muscles (CM). A Malpighian tubule (MT) is visible in the hemocoel. Scale bar = 30  $\mu$ m.

thesis, Maxwell [33] provides a line drawing of the larval digestive tract of *N. abietis* drawn from histological sections (her plate II, E) that shows the structure of the digestive tract to be similar to our observations. In Maxwell’s drawing, however, the folds in the epithelium appear to be in the middle region of the midgut, whereas we observed them at the anterior and posterior ends of the midgut (Figures 2, 9, 13, and 14). The reason for this difference is not clear, but it could be that Maxwell sectioned different larval stadia from those we sectioned, or the differences could be due to fixation artifact or it could be that the material examined by Maxwell was infected with NeabNPV. NeabNPV is highly contagious in populations of balsam fir sawfly larvae and NeabNPV can be acquired by larvae shortly after emergence from the egg and quickly transmitted to other larvae [34]. In the material we examined, columnar epithelial cells and nidi of regenerative cells were the principle cell types observed in the middle region of midgut of larval *N. abietis*. The simplicity of the larval gut of *N. abietis* is perhaps reflected

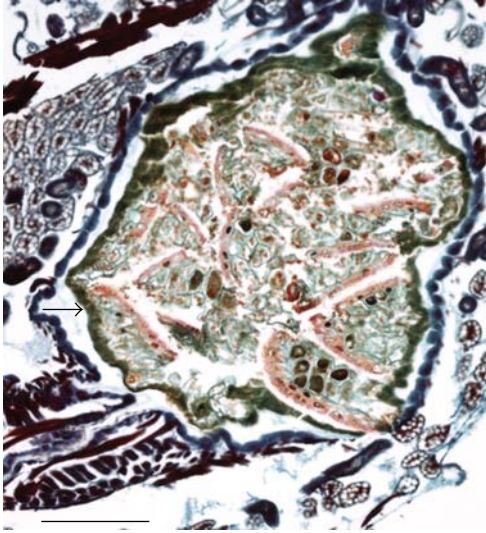


FIGURE 18: Fecal pellet surrounded by a sheath (arrow) formed from the peritrophic membrane in the bulbous rectum of a *N. abietis* larva. Scale bar = 0.2 mm.



FIGURE 19: Longitudinal section from the anus of a *N. abietis* larva showing an innervated (arrowhead) seta (S) extending from the cuticle (C), which is underlain by a single layer of epithelial cells (Ep) and fat body (FB). Posteriorly directed cuticular spines are indicated by the arrows. Scale bar = 60  $\mu$ m.

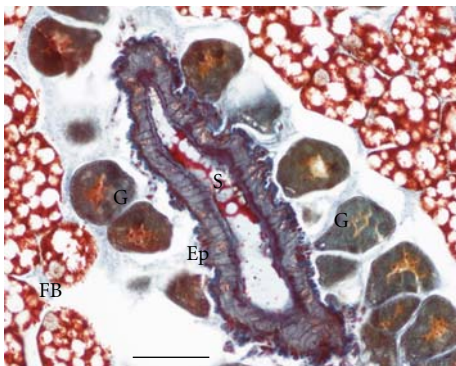


FIGURE 20: Anterior duct of larval *N. abietis* salivary gland showing the single layer of epithelial cells (Ep) and secretory products (S) in the central lumen. Gland cells (G) lie in the hemocoel adjacent to the duct and near fat body (FB). Scale bar = 0.1 mm.

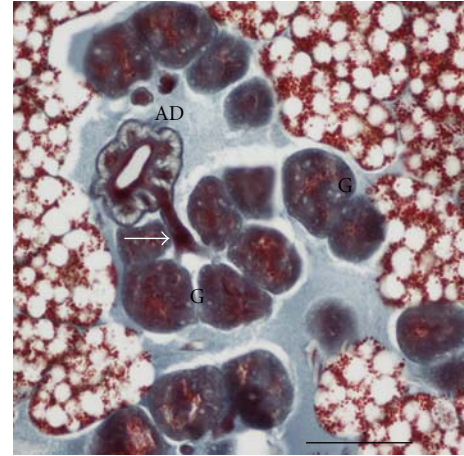


FIGURE 21: Tubule (arrow) connecting gland cells (G) to the anterior duct (AD) of a *N. abietis* larval salivary gland. Scale bar = 0.1 mm.

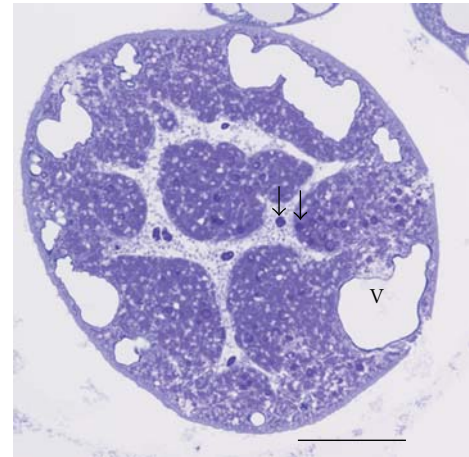


FIGURE 22: Detail of gland cell with vacuoles (V) in the cytoplasm and granules (arrows) in both the cytoplasm and the lumen. Scale bar = 30  $\mu$ m.

by the low diversity of gut bacteria as determined by culture-independent molecular techniques (i.e., polymerase chain reaction amplification and sequencing of conserved 16S rRNA genes from microbiota) [35, 36].

Santos and Serrao [37] examined the ileums of 47 species of bees and concluded that in general, the ileum is a chitin-lined tube formed by a single layer of cuboid cells with no evidence of anatomical specialization and that the ileum is surrounded by a layer of circular muscle. This would also describe the ileum of *N. abietis* larvae. The rectum of *N. abietis* larvae also appears to have a simple anatomy consisting of a single layer of epithelial cells with a thinner cuticle and fewer muscles than the ileum. Fecal pellets in the rectum were covered by peritrophic membrane. Maxwell [10] refers to “rectal teeth” patterns and the possibility of using this characteristic to distinguish different species of Diprionidae; she notes her intention to publish a paper on this subject. Unfortunately, if she published such a paper, we

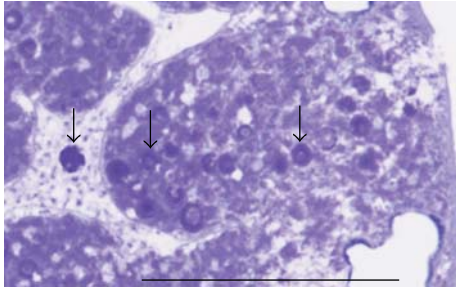


FIGURE 23: Further detail of granules (arrows) that originate from the gland cell shown in Figure 22. Scale bar = 30  $\mu\text{m}$ .

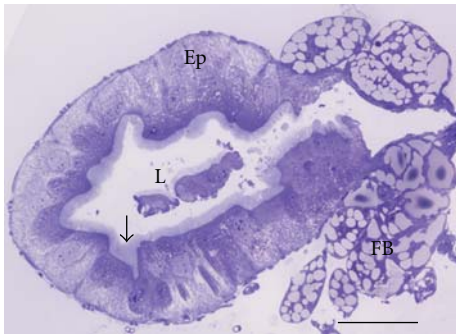


FIGURE 24: Anterior duct of a *N. abietis* larva salivary gland showing the single layer of epithelial cells (Ep) with a border of microvilli (arrow) lining the lumen (L) of the duct. Fat body (FB) cells are pressed against the duct. Scale bar = 0.1 mm.

have been unable to find it. In general, Maxwell [10] reports two rows of rectal teeth in the Diprionidae (e.g., *N. swainei*, *Diprion (Gilpinia) hercyniae*). We observed numerous, saw-like teeth and setae lining the cuticle of the anal canal of *N. abietis*, but it is unclear whether Maxwell was referring to either of these structures.

Saliva in insects serves as a lubricant for food entering the digestive tract; it may contain enzymes and may [38] or may not [31] be involved in digestion. Salivary glands of sawflies and higher Hymenoptera are labial glands. Unlike the salivary glands of sawflies, where the gland cells are clearly evident on the salivary glands [10, 39], the gland cells of higher Hymenoptera have been incorporated into the lining of the salivary ducts [40]. In addition to the production of saliva, the salivary glands of sawflies may also function as silk glands for cocoon production in some groups, such as Xyelidae, Cephidae, and Tenthredinoidea (except Blasticotomidae) but not others, for example, Pamphiliidae, Siricidae, and Xiphydriidae (see [41]). In the honey bee (*Apis mellifera*), silk production begins and ends in the fifth larval stadium prior to the prepupal period [42, 43]. Presumably, a similar process would occur in the larval stadium just prior to pupation in *N. abietis* and other diprionid sawflies.

Maxwell [10, 33] undertook her study of the internal anatomy of 132 species of sawfly larvae to determine the value of internal characteristics as indicators of taxonomic and phylogenetic relationships with the view that sawflies

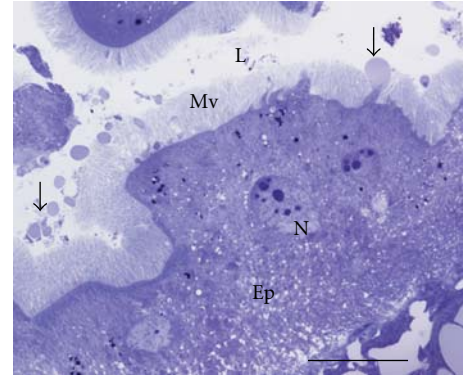


FIGURE 25: Detail of a *N. abietis* larva salivary gland anterior duct showing the nucleus (N) and microvilli (Mv) of epithelial cells (Ep) and secretory products (arrows) in the lumen (L) of the duct. Scale bar = 30  $\mu\text{m}$ .

were part of a monophyletic group, the Symphyta. More recent studies, however, indicate that the sawflies are not monophyletic [44, 45]. Instead, the different superfamilies of sawflies form branches off of the main evolutionary line from a common hymenopteran ancestor to the Euhymenoptera (Orussoidea and Apocrita) [45]. The differences in internal anatomies observed by Maxwell [10, 33] likely represent the long-standing and separate evolutionary histories of the different groups of sawflies examined. The particular relevance of an examination of the larval gut histology of a diprionid sawfly such as *N. abietis* is that the Diprionidae is the only family of sawflies, where gammabaculoviruses have been identified, isolated, and verified [46, 47]. Thus, this current paper provides information on the healthy digestive tract of a diprionid sawfly larva against which studies on the pathology of gammabaculoviruses in diprionid sawflies can be compared.

## Acknowledgments

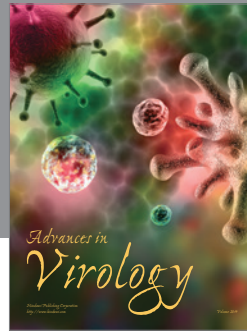
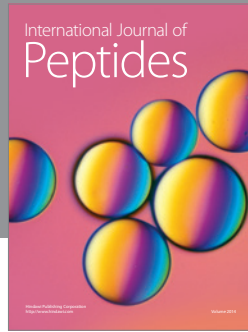
This work was supported by grants-in-aid of research to C. J. Lucarotti and D. B. Levin from the Natural Sciences and Engineering Research Council (NSERC) through an Industrial Research Partnership with Forest Protection Limited (Lincoln, New Brunswick) and the NSERC BioControl Network, and through grants from Natural Resources Canada and the Canadian Forest Service to C. J. Lucarotti. The authors gratefully acknowledge the assistance of Alison Connors in sectioning paraffin-embedded material and Rob Johns and Renée Lapointe for critical reviews of the paper.

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