# Phoretic mite associates of mountain pine beetle at the leading edge of an infestation in northwestern Alberta, Canada

**Boyd A. Mori, Heather C. Proctor, David E. Walter, Maya L. Evenden**

**Abstract**—We identified species of mites phoretically associated with mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), collected from bolts of lodgepole pine, *Pinus contorta* Douglas *ex* Louden (Pinaceae), and pheromone-baited traps in northwestern Alberta, Canada. Mite load and species composition were compared between beetle sexes and with beetle emergence time and estimated body size. The vast majority of mites associated with *D. ponderosae* in Alberta belonged to three species: *Proctolaelaps subcorticalis* Lindquist (Acari: Mesostigmata: Melicharidae), *Histiogaster arborsignis* Woodring (Acari: Astigmatina: Acaridae), and *Tarsonemus ips* Lindquist (Acari: Prostigmata: Tarsonemidae). There was no difference in mite loads on male and female beetles recovered from bolts in the laboratory and those from pheromone-baited traps in the field. More mites were found on larger beetles in the laboratory, but only *T. ips* showed this pattern on field-trapped beetles. There was no relationship between total mite load or load by mite species and beetle emergence time in the laboratory, but total mite load on field-trapped beetles decreased over the collecting season (10 June – 3 September 2009) at five collection locations (Grovedale, Blueberry Mountain, Hythe, Evergreen Park, and Glenleslie). This study is the first to document the assemblage of phoretic mites on *D. ponderosae* in Alberta and will help to direct future research on their interactions.

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**Résumé**—Nous avons identifié les espèces d'acariens phorétiques sur des dendroctones du pin ponderosa, *Dendroctonus ponderosae* Hopkins (Coleoptera : Curculionidae : Scolytinae), récoltés sur des billes de pin vrillé, *Pinus contorta* Douglas *ex* Louden (Pinaceae), et dans des pièges munis de phéromones dans le nord-ouest de l'Alberta, Canada. Les charges d'acariens et les compositions en espèces ont été comparées chez les coléoptères des deux sexes en fonction du moment de l'émergence et de la taille corporelle estimée. La grande majorité des acariens associés à *D. ponderosae* en Alberta appartiennent à trois espèces, *Proctolaelaps subcorticalis* Lindquist (Acari : Mesostigmata : Melicharidae), *Histiogaster arborsignis* Woodring (Acari : Astigmatina : Acaridae) et *Tarsonemus ips* Lindquist (Acari : Prostigmata : Tarsonemidae). Il n'y a pas de différence de charge d'acariens entre les coléoptères mâles et femelles prélevés sur les billes en laboratoire, ni dans les pièges à phéromones en nature. Plus d'acariens se retrouvent en laboratoire sur les coléoptères plus grands, mais seul *T. ips* suit ce patron sur les coléoptères dans les pièges. Il n'y a pas de relation entre la charge totale d'acariens ou entre la charge en fonction des espèces d'acariens et le moment de l'émergence des coléoptères en laboratoire; cependant, la charge totale d'acariens sur les coléoptères capturés dans les pièges en nature diminue au cours de la saison de récolte (10 juin – 3 septembre 2009) aux cinq sites de capture (Grovedale, Blueberry Mountain, Hythe, Evergreen Park et Glenleslie). Notre étude est la première à caractériser le peuplement d'acariens phorétiques sur *D. ponderosae* en Alberta et aidera à orienter les recherches futures sur leurs interactions.

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## Introduction

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), is a major pest of pine forests throughout western North America (Safranyik and Carroll 2006). In recent years a major outbreak has caused extensive damage in British Columbia, Canada, and continues to spread eastward into Alberta (Ono 2003). *Dendroctonus ponderosae* can attack and kill most exotic and native pine species (*Pinus* L.: Pinaceae) in North America (Amman and Cole 1983; Ono 2003). In Alberta this includes healthy mature lodgepole (*Pinus contorta* Engelmann), limber (*P. flexilis* James), and whitebark (*P. albicaulis* Engelmann) pines (Ono 2003). *Dendroctonus ponderosae* also can establish on jack pine (*P. banksiana* Lambert) (Cerezke 1995), and has successfully reproduced in hybrid spruce *Picea engelmannii* × *glauca* in British Columbia (Huber *et al.* 2009). The beetle has the potential to spread across Canada through the boreal forest, which may have devastating consequences for the Canadian timber industry (Cerezke 1995; Ono 2003). From an ecological viewpoint, expansion of the *D. ponderosae* outbreak into Alberta provides opportunities for establishment of new relationships among the beetle, its tree hosts, and the fungi, insects, mites, and other organisms associated with the pine subcortical habitat.

Phoretic mites are often found on bark beetles and other insects (Moser and Roton 1971; Rodrigueiro and Do Prado 2004; Cardoza *et al.* 2008; Grossman and Smith 2008). Phoresy, the use by a small animal of a larger animal for transport, is a common life-history strategy for mites living in patchy, ephemeral habitats (Binns 1982; Rocha *et al.* 2009). Phoretic relationships may be commensalistic or mutualistic but may also have negative consequences for the host (Walter and Proctor 1999; Hofstetter *et al.* 2006*a*; Grossman and Smith 2008). Phoretic mites can directly reduce flight velocity of individuals of *Ips* De Geer (Coleoptera: Curculionidae: Scolytinae) species and prey on their eggs in galleries after transport to the new environment (Lindquist and Bedard 1961; Kinn and Witcosky 1978). Additionally, the presence of clusters of phoretic mites at the tips of host elytra reduces wing-beat frequency of Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopkins (Atkins 1960). A reduction in flight velocity or wing-beat frequency may indirectly harm bark beetles through decreased dispersal and colonization.

Many phoretic mites on bark beetles consume the mutualistic fungi associated with the beetles. Bark beetle hosts transport mites to habitats where the beetles introduce fungi upon which the mites feed (Lombardero *et al.* 2000; Rocha *et al.* 2009; Moser *et al.* 2010). Some tarsonemid mites not only feed on but also directly vector many blue-stain fungi associated with bark beetles (Lombardero *et al.* 2003; Moser *et al.* 2010). In contrast, some species of *Tarsonemus* Canestrini and Fanzago transport the fungus *Ophiostoma minus* Hedgecock, Hunt and P. Sydow (Ophiostomataceae), which is antagonistic to the southern pine beetle, *Dendroctonus frontalis* Zimmerman, and its mycangial fungi (mycangia are specialized structures on the beetle integument for active transport of symbiotic fungi) (Moser 1985; Lombardero *et al.* 2000; Hofstetter *et al.* 2007). Colonization of the phloem by *O. minus* inhibits egg production, reduces larval growth, and lowers larval survival in *D. frontalis*, and *O. minus* may compete for phloem with beneficial mycangial fungi introduced by the beetle (Barras 1970; Franklin 1970; Klepzig and Wilkens 1997). Phoretic mites on *Scolytus* Geoffroy spp. help vector the fungus responsible for Dutch elm disease, *Ophiostoma novo-ulmi* Brasier (Moser *et al.* 2010).

Ecological interactions between bark beetles and their phoretic mites in other bark beetle systems suggest that analogous relationships may occur in the *D. ponderosae* system, but so far these relationships have not been studied. There are only a few records of mites on *D. ponderosae* (Lindquist and Hunter 1965; Lindquist 1969, 1971; Moser and Roton 1971). Given the economic importance of *D. ponderosae*, this lack of research is surprising, as extensive literature exists on phoretic mites associated with *D. frontalis* (Moser 1976; Kinn and Witcosky 1978; Lombardero *et al.* 2000; Hofstetter *et al.* 2007), the spruce beetle, *Dendroctonus rufipennis* Kirby (Cardoza *et al.* 2008), species of *Pityokteines* Fuchs (Pernek

*et al.* 2008) and *Scolytus* (Moser *et al*. 2010), and *Ips typographus* L. (Takov *et al*. 2009).

Herein we identify the species of mites associated with *D. ponderosae* from northwestern Alberta and determine how mite load and species composition vary with beetle emergence date, sex, and estimated body size. This information will begin to document the relationships between *D. ponderosae* and its phoretic mites in Alberta and help to direct future studies exploring the relationships between the beetles, mites, and fungi.

## Materials and methods

### Laboratory study

In March 2008, two *D. ponderosae*-infested lodgepole pine trees were felled 100 km southwest of Grande Prairie, Alberta (54.69°N, 119.02°W). One 60 cm long bolt was cut from each tree 1 m above the soil surface. Bolts were transported to the laboratory at the University of Alberta and the cut ends were sealed with paraffin wax to prevent desiccation before storage at 4 $\pm$ 2 °C until use. In August 2008 the two bolts were placed at room temperature (22 $\pm$ 2 °C) in separate 121 L bins made of opaque plastic and fitted with emergence jars. Beetle emergence was monitored daily and collected beetles were separated by sex (Lyon 1958). Pronotum width and body length of each beetle were measured to the nearest 0.08 mm using an ocular micrometer on a dissecting microscope (12 × magnification). From these measurements, the approximate surface area of an ellipsoid (Knud Thomsen formula: $S \approx 4\pi[a^p b^p + a^p c^p + b^p c^p]^{\frac{1}{p}}$) was calculated for each beetle ($a = b$ = half of pronotum width, $c$ = half of length, and $p = 1.6075$) to estimate beetle size (Michon 2009; Xu *et al.* 2009). After measurements were made, beetles were placed individually in 1.5 mL microfuge tubes and preserved in 75% ethanol.

Twenty beetles per emergence day were randomly selected from each bolt and the phoretic mites on each beetle removed. Mites were found floating with the beetle in the ethanol or were physically removed from the beetles using forceps. The elytra of each beetle were lifted and the undersides of the elytra, hind wings, and dorsal surface of the abdomen were checked for mites. Mites were slide-mounted in No. 6371A polyvinyl alcohol mounting medium (Bioquip, St. Rancho Dominguez, California, United States of America) and identified using differential interference microscopy at up to 1000 × magnification. The taxonomic literature included Woodring (1966), Lindquist and Hunter (1965), Lindquist (1969), Magowski and Moser (2003), and keys constructed by D.E.W. A few specimens were examined using scanning electron microscopy after dehydration through an ethanol series (85%, 90%, 95%, absolute), dried using a Bal-Tec CPD 030 critical-point dryer, mounted on stubs, and sputter-coated with gold using a Nanotech SEMPrep2 set at 120Å. Scanning electron micrographs were taken in the Department of Earth and Atmospheric Sciences, University of Alberta, with a JEOL 6301F scanning electron microscope at 5 kV. Exemplars of mite taxa identified in this study have been deposited in the E.H. Strickland Entomological Museum at the University of Alberta and in the Royal Alberta Museum, Edmonton.

### Field study

In June 2009, five study sites were established in lodgepole pine stands around Grande Prairie, Alberta (55.17°N, 118.80°W). At each site, three 12-unit Lindgren funnel traps (Contech Enterprises Inc., Delta, British Columbia) baited with *D. ponderosae* lures consisting of *trans*-verbenol, *exo*-brevicomin, and myrcene (Contech Enterprises Inc.) on steel-rod stands were positioned along a linear transect at 25 m intervals and 5 m from the nearest tree, with the bottom of the trap approximately 0.5 m above the soil surface. Ethylene glycol (Prestone 50/50 prediluted antifreeze/coolant, Canadian Tire Corporation, Ltd., Edmonton) was used as a killing agent and preservative. Traps were checked weekly throughout the beetle flight period from 18 June to 3 September 2009. At each date, the contents of the traps were poured into glass jars and the antifreeze in the traps was replenished. Specimens were transported to the laboratory for sorting and analyses.

*Dendroctonus ponderosae* recovered from each trap at each collection date were placed individually in 1.5 mL microfuge tubes and

preserved in 75% ethanol. Twenty beetles per trap and collection date were examined for mites from six collection periods throughout the beetle flight period in 2009: (1) 18–19 June; (2) 3–5 July; (3) 14–16 July; (4) 21–24 July; (5) 4–5 August; (6) 1–3 September. Beetles were separated by sex and then pronotum width and body length of each beetle were measured to the nearest 0.08 mm using an ocular micrometer on a dissecting microscope (12 × magnification). From these measurements the approximate surface area of an ellipsoid was calculated as in the laboratory study. Only mites found directly on beetles were considered, as a large number of non-*D. ponderosae* insects were collected in the field study, which may have contributed to the mites found floating in the preservative. Mites were removed from beetles, slide-mounted, and identified as in the laboratory study. Exemplars of mites identified in the field study have been deposited in the E.H. Strickland Entomological Museum and the Royal Alberta Museum.

### Data analyses

Prior to analyses, data were tested for normality using a Shapiro–Wilk $W$ test, and non-normal data were transformed as necessary. Beetle surface area data were transformed with a natural-logarithm transformation, and mite count data with a cube-root transformation to approach the assumptions of normality. In the laboratory study, to test the hypothesis that total mite load and the load of each mite species varied with beetle sex and surface area, factorial analysis of variance (ANOVA) was conducted using a general linear model (GLM) approach (SYSTAT® 12.0; SAS Institute Inc. 2007, Chicago, Illinois). Models tested included total mite load regardless of mite species or individual mite species load as the dependent variable, with beetle sex, beetle surface area, and sex × surface area specified as the independent variables, and bolt treated as a blocking factor. Surface areas of male and female beetles were compared using a randomized block ANOVA with bolt specified as the block (SYSTAT® 12.0). The hypotheses that beetle surface area is related to total mite load and to the load of each mite species were tested with ordinary least squares (OLS) linear regression analyses (SYSTAT® 12.0). Beetles that did not have mites were excluded from these analyses. To determine whether beetle emergence day was related to mite load and the load of each mite species, OLS linear regression analysis was used (SYSTAT® 12.0) on all beetles that emerged from bolts. Analyses of mite load by emergence day were conducted separately for beetles from each bolt, owing to the variation in emergence time between bolts.

In the field experiment, the distributions of surface areas of beetles with and without mites were compared using a two-sample $t$ test. As these distributions did not differ ($t = 0.388$, $P = 0.698$, df = 250), two-factor factorial ANOVAs using a GLM approach were conducted only on beetles that carried mites, to test for differences in total mite load and the load of each mite species with beetle sex and beetle surface area, as in the laboratory study (SYSTAT® 12.0). The surface areas of male and female beetles were compared using a one-way ANOVA (SYSTAT® 12.0). OLS linear regression analyses tested whether beetle surface area and flight period predicted total mite load and the load of each mite species, respectively (SYSTAT® 12.0).

Sampled beetles from both the laboratory and the field study were divided into four categories based on mite load. The proportions of beetles in each category in the field and laboratory studies were compared using $\chi^2$ analysis (SYSTAT® 12.0). To determine whether the relative proportions of the three major mite taxa (*Proctolaelaps subcorticalis* Lindquist (Acari: Mesostigmata: Melicharidae), *Histiogaster arborsignis* Woodring (Acari: Astigmatina: Acaridae), and *Tarsonemus ips* Lindquist (Acari : Prostigmata : Tarsonemidae); see Results) differed between the laboratory and field studies, a $\chi^2$ analysis was used (SYSTAT® 12.0).

## Results

### Laboratory study

Throughout the emergence period, 473 beetles from two bolts were examined and 2640 beetle-associated mites were recovered (Table 1). Four species of mites from four families were recovered from beetles reared from bolts under laboratory conditions. Three of these were very

Table 1. Numbers of different mite taxa found on male and female *Dendroctonus ponderosae* and percentages of *D. ponderosae* carrying each taxon in the laboratory and field studies.

| | No. of mites on *D. ponderosae* | | Percentage of *D. ponderosae* carrying the mite taxon | |
|---|---|---|---|---|
| Mite | Males | Females | Males | Females |
| **Laboratory study** | | | | |
| *Proctolaelaps subcorticalis* | 217 | 673 | 49.0 | 59.9 |
| *Histiogaster arborsignis* | 517 | 780 | 71.7 | 70.9 |
| *Tarsonemus ips* | 152 | 279 | 33.2 | 32.9 |
| Tydeidae | 1 | 0 | 0.54 | 0.0 |
| **Field study** | | | | |
| *Proctolaelaps subcorticalis* | 156 | 257 | 14.1 | 12.5 |
| *Histiogaster arborsignis* | 17 | 13 | 3.8 | 2.2 |
| *Tarsonemus ips* | 117 | 175 | 19.3 | 18.6 |
| *Proctolaelaps* spp. | 0 | 1 | 0.0 | 0.20 |
| *Macrocheles schaeferi* | 0 | 1 | 0.0 | 0.20 |

common: *P. subcorticalis* (Fig. 1*a*), *H. arborsignis*, and *T. ips* (Fig. 1*b*). In addition, we recovered one specimen of an unidentified species in the family Tydeidae (Prostigmata).

The number of mites associated with individual beetles varied considerably (Fig. 2). Multiple mite species were found to associate with individual beetles. In the laboratory study, one or two mite species were most often found on individual beetles (Fig. 3*a*). Female beetles had a significantly larger body than males ($F_{1,470} = 132.377$, $P < 0.001$) and beetles with a larger body had a higher mite load ($F_{1,469} = 9.842$, $P = 0.002$); however, total mite load did not differ according to beetle sex ($F_{1,469} = 0.491$, $P = 0.484$). The numbers of *P. subcorticalis* ($F_{1,469} = 2.122$, $P = 0.146$), *H. arborsignis* ($F_{1,469} = 1.777$, $P = 0.183$), and *T. ips* ($F_{1,469} = 0.083$, $P = 0.774$) were equally distributed between the two sexes of beetle. Beetle size was significantly related to total mite load for beetles that carried mites ($R^2 = 0.023$, $n = 425$, $P = 0.002$), but did not account for much of the variation in mite load per beetle. Individual regression analyses by mite species revealed a significant positive relationship with beetle surface area for *P. subcorticalis* ($R^2 = 0.026$, $n = 425$, $P = 0.001$) and *H. arborsignis* ($R^2 = 0.011$, $n = 425$, $P = 0.030$) on individual beetles; however, there was no relationship for *T. ips* ($R^2 < 0.001$, $n = 425$, $P = 0.929$). Beetle emergence day was not related to total mite load (bolt 1: $R^2 = 0.012$, $n = 196$, $P = 0.068$; bolt 2: $R^2 = 0.001$, $n = 277$, $P = 0.261$). The pattern of emergence of *P. subcorticalis* and *H. arborsignis* followed that of beetle emergence; the less numerous *T. ips* peaked later in the emergence period (Fig. 4*a*).

## Field study

Throughout the flight period, 915 beetles were examined from pheromone-baited traps and 818 beetle-associated mites were recovered from 276 beetles (Table 1). The same three dominant mite species were identified from field-caught beetles as in the laboratory experiment: *P. subcorticalis*, *H. arborsignis*, and *T. ips*. In addition, five other mite taxa were identified: two *Schizosthetus* cf. *lyriformis* McGraw and Farrier (Mesostigmata: Parasitidae), singletons of *Macrocheles schaeferi* Walter (Mesostigmata: Macrochelidae), a species each of *Histiostoma* Kramer (Astigmatina: Histiostomatidae) and *Leptus* Latreille (Prostigmata: Erythraeidae), and a species of *Proctolaelaps* Berlese different from *P. subcorticalis*. The specimens of *Histiostoma* sp., *Leptus* sp., and *Proctolaelaps* sp. could not be identified to species, owing to their poor quality.

The number of mites associated with an individual beetle varied considerably, but most beetles sampled from pheromone-baited traps had no attached mites (Fig. 2). Multiple mite species were found to associate with individual

**Fig. 1.** (*a*) Scanning electron micrograph of *Proctolaelaps subcorticalis* (dorsal view). The arrow indicates fungal conidia attached to the exoskeleton. (*b*) Scanning electron micrograph of *Tarsonemus ips* (ventral view). The arrows indicate flap-like sporothecae for carrying fungal conidia.

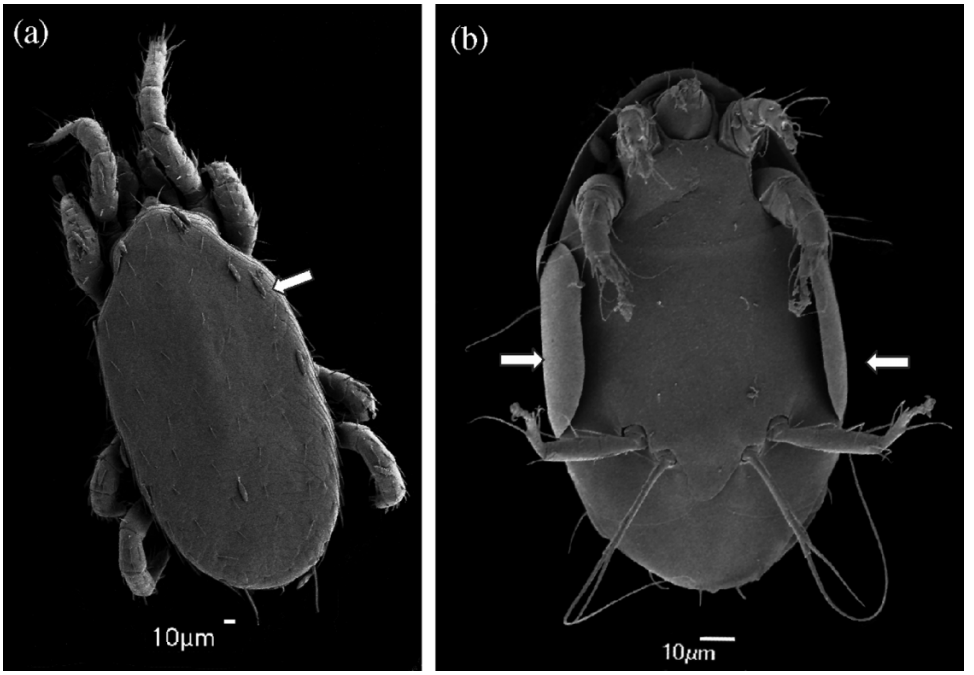
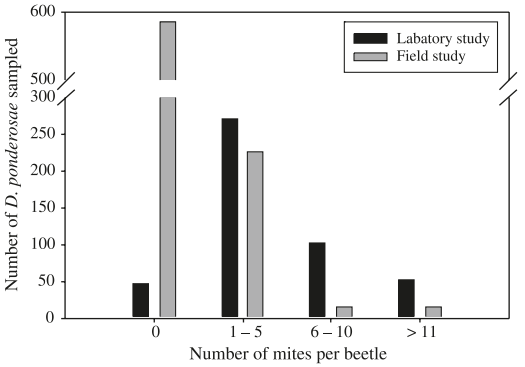



**Fig. 2.** Densities of mites found on *Dendroctonus ponderosae* in the laboratory and field studies ($\chi^2$ = 477.30, df = 3, $P < 0.001$).




beetles captured in pheromone-baited traps; however, most trap-captured beetles with mites carried only one species (Fig. 3*b*). When only those beetles that did have mites were considered, there was no effect of beetle sex on total mite load ($F_{1,66}$ = 0.004, $P$ = 0.950) or the number of each species (*P. subcorticalis*: $F_{1,66}$ = 0.026, $P$ = 0.873; *H. arborsignis*: $F_{1,66}$ = 1.272, $P$ = 0.264; *T. ips*: $F_{1,66}$ = 0.342, $P$ = 0.561). Female beetles recovered from pheromone-baited traps had a larger body than trapped males ($F_{1,67}$ = 13.241, $P$ = 0.001), which is consistent with the results of the laboratory study; however, beetle body size was not related to total mite load in the field study ($R^2 < 0.001$, $n$ = 69, $P$ = 0.495). Regression analyses by individual mite species showed that beetle body size was not related to the number of *P. subcorticalis* ($R^2$ = 0.024, $n$ = 69, $P$ = 0.109) or *H. arborsignis* ($R^2 <$ 0.001, $n$ = 69, $P$ = 0.940) on beetles, but was weakly related to the number of *T. ips* ($R^2$ = 0.065, $n$ = 69, $P$ = 0.019).

The time of sampling period during the flight season significantly predicted the total phoretic mite load on beetles in the field study ($R^2$ = 0.395, $n$ = 842, $P < 0.001$; Fig. 5) and indicated that mite load in general decreased slightly over the flight season. Individual mite species were present at different times during beetle flight (Fig. 4*b*). *Proctolaelaps subcorticalis* were present throughout the entire beetle

**Fig. 3**. Numbers of mite species present on individual *Dendroctonus ponderosae* in the laboratory study (the results for one of two bolts are shown) (*a*) and the field study (*b*).

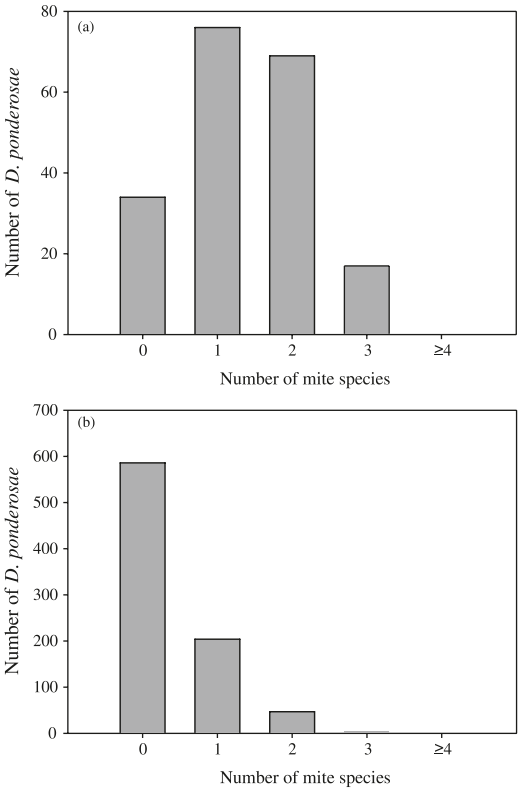
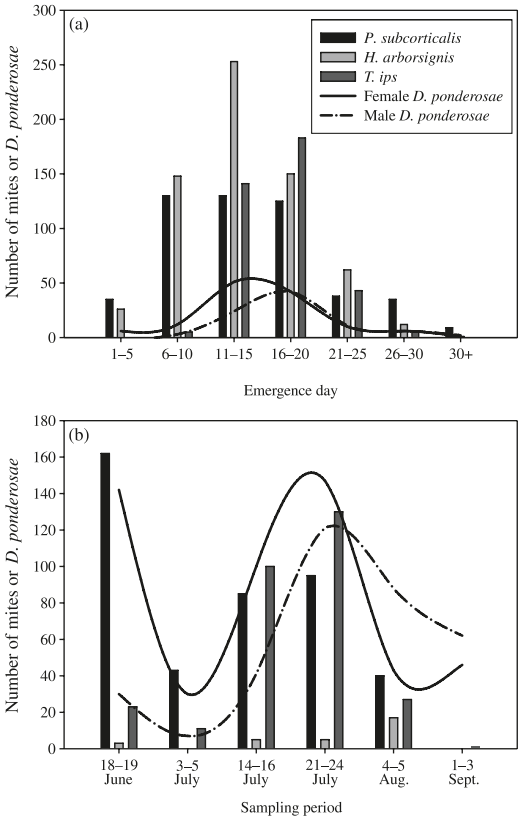



**Fig. 4**. *Dendroctonus ponderosae* and mite emergence over time in the laboratory study (the results for one of two bolts are shown) (*a*) and field study (*b*).




flight period, whereas *H. arborsignis* were infrequently found on beetles recovered from pheromone-baited traps. The number of *T. ips* followed beetle abundance patterns throughout the flight period (Fig. 4*b*).

Overall, mite load on sampled beetles differed significantly between the laboratory and field studies ($\chi^2 = 477.30$, df = 3, $P < 0.001$; Fig. 2). As well, the relative proportions of each mite species differed significantly between the two studies on both female ($\chi^2 = 293.48$, df = 2, $P < 0.001$) and male beetles ($\chi^2 = 243.07$, df = 2, $P < 0.001$) (Fig. 6).

## Discussion

In the laboratory and field studies, we found three species of phoretic mites commonly associated with *D. ponderosae* in Alberta: *P. subcorticalis*, *H. arborsignis* and *T. ips*. This diversity is low compared with that in assemblages of phoretic mites found on beetles in other species of *Dendroctonus* Erichson (Moser and Roton 1971; Cardoza *et al.* 2008) and may indicate recently established relationships on the leading edge of the *D. ponderosae* outbreak spreading into Alberta. These mite species have been reported widely across North America in association with other bark beetles, including *D. frontalis* (Hofstetter *et al.* 2007), *D. rufipennis* (Cardoza *et al.* 2008), and *Ips avulsus* Eichoff (Moser and Roton 1971). These species of phoretic mites may have been transported to Alberta during beetle dispersal or may have adopted *D. ponderosae* as a host since its arrival in Alberta. *Proctolaelaps subcorticalis* has previously been recorded on another scolytine, *Trypodendron lineatum* (Olivier), in Alberta

**Fig. 5**. Linear regression of cube-root-transformed total numbers of mites per field-captured individual *Dendroctonus ponderosae* during each sampling period [$y = 1.618 - 0.290(\text{time})$] ($R^2 = 0.395$, $n = 842$, $P < 0.001$).

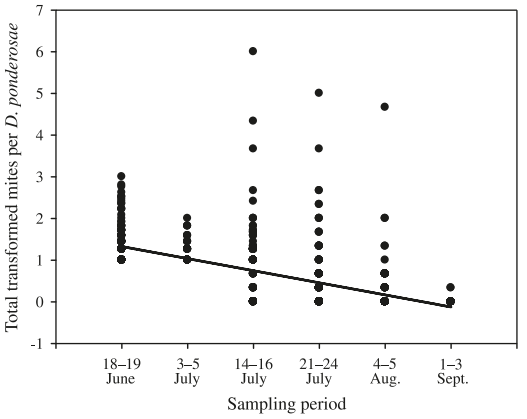
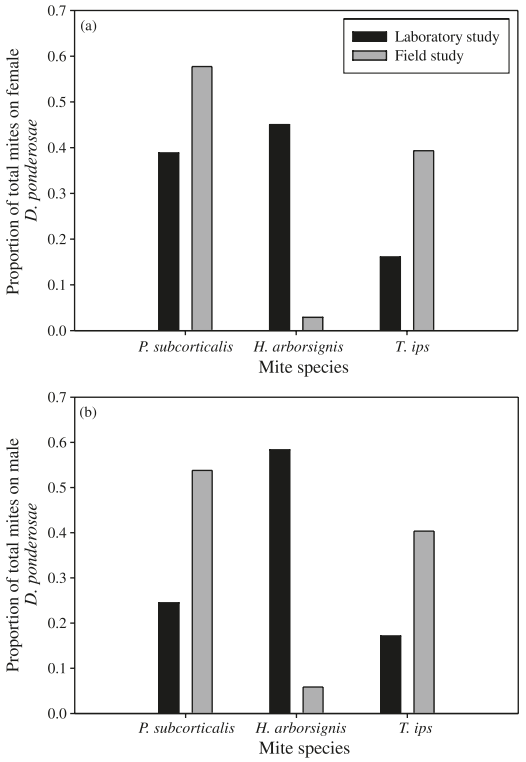



**Fig. 6**. Proportions of the three predominant mite species obtained in the laboratory and field studies from female ($\chi^2 = 293.48$, df = 2, $P < 0.001$) (*a*) and male *Dendroctonus ponderosae* ($\chi^2 = 243.07$, df = 2, $P < 0.001$) (*b*).




(Lindquist 1971). There are existing records for both *P. subcorticalis* and *T. ips* from Alberta and British Columbia (Lindquist 1969). A population-genetic analysis of phoretic mites associated with *D. ponderosae* in Alberta and source populations in British Columbia is needed to clarify the origin of these mites.

*Proctolaelaps subcorticalis* occurs in most temperate regions of North America and appears to be restricted to the subcortical habitat associated with scolytine beetles in dead and dying trees (Lindquist and Hunter 1965; Lindquist 1971). There are two previous records of *Proctolaelaps* spp. on *D. ponderosae*: *Proctolaelaps hystricoides* Lindquist and Hunter in California and *P. subcorticalis* from Radium, British Columbia (Lindquist and Hunter 1965; Lindquist 1971). Other *Proctolaelaps* species, including *P. dendroctoni* Lindquist and Hunter, are predatory on nematodes and on tritonymphs of *H. arborsignis* (Lindquist and Hunter 1965; Moser and Roton 1971; Kinn 1983). *Proctolaelaps subcorticalis* may benefit *D. ponderosae* by preying on nematodes that negatively affect it , decreasing their fecundity and dispersal capacity and increasing adult mortality (Safranyik and Carroll 2006). *Proctolaelaps subcorticalis* may also affect subcortical-community interactions through the transportation of fungi as has been demonstrated for other mites that are phoretic on bark beetles (Moser 1985; Cardoza *et al.* 2008; Moser *et al.* 2010). We observed fungal spores attached directly to the cuticle of *P. subcorticalis* (Fig. 1*a*), which suggests that it vectors the fungi between trees.

*Histiogaster* spp. are found on a variety of wood-boring insects and in galleries constructed by scolytine beetles (OConnor 1994). The large majority of *Histiogaster* species, including *H. arborsignis*, are generalist fungivores (Moser and Roton 1971; OConnor 1990; Cardoza *et al.* 2008). In laboratory experiments, *H. arborsignis* also preyed on the larvae of *D. frontalis* (Moser 1975) and eggs of *D. rufipennis*, but these results were confounded with conditions that were unfavourable for beetle development (Cardoza *et al.* 2008).

*Histiogaster* spp. carry fungal spores on their bodies (Cardoza *et al.* 2008) and therefore *H. arborsignis* on *D. ponderosae* may vector and feed on the mutualistic fungi associated with the beetle or on other fungi in the environment.

*Tarsonemus ips* is fungivorous and has flap-like structures, sporothecae, to carry fungal spores (Fig. 1*b*, arrows) (Moser 1985; Moser *et al.* 2010). *Tarsonemus ips* associated with *D. frontalis* carry *O. minus*, an antagonistic fungus that reduces beetle fecundity and larval development (Moser 1985; Moser and Bridges 1986; Lombardero *et al.* 2000; Hofstetter *et al.* 2006*b*). The species of fungi carried by *T. ips* in the current study are unknown, because mites were preserved in ethanol and fungal spores could not be cultured. Identification of the fungal spores associated with *T. ips* would help to clarify the role of this mite in the subcortical community and its ecological relationship with *D. ponderosae*.

In the laboratory and field studies, mite load on *D. ponderosae* showed no sex bias. Male-biased association of phoretic mites on insect hosts occurs in some species of flies (Gilburn *et al.* 2009). In our laboratory study, mites attached to *D. ponderosae* with a larger body regardless of beetle sex. In the field study, there was no difference in mite loads on male and female beetles caught in pheromone-baited traps. Similarly, phoretic mites were evenly distributed among male and female *D. frontalis* recovered from sticky traps in the field (Moser 1976).

For *D. ponderosae*, body size was related to total mite load in the laboratory but not in the field. *Proctolaelaps subcorticalis* and *H. arborsignis* were more abundant on larger beetles in the laboratory, whereas *T. ips* showed no partiality; in contrast, the load of *T. ips* in the field was positively associated with beetle body size. More phoretic mites were found on larger *Nicrophorus investigator* Zetterstedt (Coleoptera: Silphidae) beetle hosts that are better able to locate a breeding resource (Grossman and Smith 2008). Larger *D. ponderosae* have more fat reserves and disperse farther (Pureswaran and Borden 2003), and so may transport phoretic mites a greater distance from the natal habitat than can smaller beetles. Alternatively, mites may attach to larger beetles simply because there are more attachment sites, or because the probability of encountering larger hosts is higher. Although the relationships between mite load and body size in this study were significant in several instances, they explained little variation and their biological significance is likely limited.

Mite load per beetle decreased slightly but significantly over the flight period in the field; however, there was no relationship between mite load and emergence time on beetles in the laboratory study. In the field, mites may attach to beetles early in the flight season to escape from a deteriorating habitat. *Proctolaelaps subcorticalis* were abundant on beetles recovered from pheromone-baited traps throughout the flight period (Fig. 4*b*), whereas *T. ips* appeared on beetles later in the flight season and on later-emerging beetles in the laboratory (Fig. 4*b*). *Tarsonemus ips* may require more time in the natal habitat to load sporothecae with fungi, and therefore attach to phoretic hosts later than other mites. Alternatively, *T. ips* may synchronize their development with *D. ponderosae* as occurs in phoretic species of the closely related genus *Tarsonemoides* Tragardh on their *Ips* spp. hosts (Lindquist and Bedard 1961).

The majority of beetles recovered from pheromone-baited traps did not carry phoretic mites, but almost all beetles that emerged from bolts in the laboratory had mites (Fig. 2). The proportions of beetles carrying different categories of mite load differed significantly between the laboratory and field studies (Fig. 2). This may indicate that laboratory conditions are favourable for mite development and (or) that mites may be dislodged during beetle flight or removed in the antifreeze killing agent in pheromone-baited traps in the field. These factors may differentially affect particular mite species because the proportions of the three major species found on *D. ponderosae* in Alberta differed significantly between the laboratory and field studies (Fig. 6). Hofstetter *et al.* (2007) found that temperature correlated with species-specific mite loads, resulting in differences in mite assemblages associated with *D. frontalis* under different environmental conditions. The temperature in the emergence bins in our laboratory study was fairly constant at $22 \pm 2\,^{\circ}C$, whereas the mites on field-caught

beetles were exposed to a range of temperatures. Another explanation for the difference between the laboratory and field studies is that in the laboratory study only two bolts from two trees were used to obtain mites, while there may have been a large variation in the number of mites among trees.

This study is the first to document relationships between *D. ponderosae* and its phoretic mites in Alberta. Complex relationships between the beetles, mites, nematodes, and fungi present in the system are likely to occur. Mites may interact with beetles by carrying either mutualistic or antagonistic fungi. They may have a mutualistic relationship with their hosts through predation on nematodes that would otherwise harm the beetles. Mites may be opportunistic and only periodically associate with *D. ponderosae* when it is present. To determine the nature of these relationships, more research is needed to better understand the biology and interactions of *D. ponderosae* and its phoretic mite associates.

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