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Phoretic mites and their hyperphoretic fungi associated with flying *Ips typographus japonicus* Niijima (Col., Scolytidae) in Japan

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Abstract: Flying *Ips typographus japonicus* from Hokkaido (Japan) carried 12 species of phoretic mites, three of which were not previously recorded in Europe. The mite biologies were diverse, including specialists feeding on microorganisms, beetle eggs, and nematodes which were common under beetle elytra. Hyperphoretic on these mites were seven distinct species of fungal spores, plus an undetermined number identifiable only as conidia. The spores stuck anywhere on the mite bodies with no special carrying structures evident. *Ophiostoma bicolor* was the most common species, with the pathogenic *Ceratocystis polonicum* present in small numbers.

1 Introduction

The spruce beetle (SBB), *Ips typographus japonicus* (Niijima), is a trans-palearctic species occurring mainly in spruce forests but rarely in pine, fir, and larch (Wood and Bright, 1992). In Hokkaido, Japan, it is the most destructive pest of *Picea jezoensis* (Siebold and Zucc.) Carriere and *P. glehnii* (F. Schmidt) Masters. This insect is usually endemic, normally killing only over-matured or physiologically weakened trees. But when trees are harvested, SBB builds up in the slash and kills neighbouring trees for about 3 years thereafter (Koizumi, 1977); yet their overall population level does not seem to increase greatly. Heavy winds usually precede SBB outbreaks, the largest of which was seen between 1956 and 1959 after a strong typhoon in 1954 (Yamaguchi, 1963; Yamaguchi et al., 1963).

Thirty-eight species of phoretic mites have been recorded from flying SBB in Germany and Sweden (Moser and Bogenschütz, 1984; Moser et al., 1989a). Whereas the biology of most of these mite species is unknown, it is clear from studies of related bark beetles that the subcortical roles of SBB phoretic mites are quite diverse, including parasitoids, predators, filter feeders, as well as mycetophagous, nematophagous and generalist feeders (Stephen et al., 1993). In addition, these phoretic mites may transmit microorganisms in the form of hyperphoretic ascospores, tree pathogens (Moser et al., 1989b) and/or nutritional fungi vital to the survival of the beetle (Moser et al., 1995). These intimate relationships suggest that the phoretic mite fauna as well as their associated fungi coevolved with the SBB, and play an integral role in its biology. For these reasons, it is important that any studies concerning the epidemiology of this destructive bark beetle include ascertaining the roles of phoretic mites as well as their hyperphoretic fungi.

In 1981, typhoon number 15 extensively damaged forests in the northern Japanese island of Hokkaido, precipitating a buildup of SBB populations in stands of *Picea jezoensis* (Furuta et al., 1985). This event enabled us to collect SBB and compile a list of the Japanese phoretic mite species and their hyperphoretic fungal ascospores, and compare these with those collected from Europe.

2 Material and methods

Flying SBB were trapped during the months of May, June and July, 1986. Four Borregaard drain-pipe traps were deployed every 150 m along the margin of the 1981 blowdown area in the Tokyo University Forest in Furano, Hokkaido, Japan (Furuta et al., 1985). Each trap contained a dispenser containing 1500 mg methylbutenol, 70 mg cis-verbenol and 15 mg ipsdienol. Three vials containing a total of 156 beetles preserved in alcohol were sent to the senior author at the Alexandria Forestry Centre at Pineville, Louisiana. The species and number of mites as well as their hyperphoretic fungal spores were tallied from 10 beetles drawn at random from each month. Time and labour restraints dictated this small sample size. In addition, the sediment at the bottom of the collection vials was searched for any mites that may have detached from the beetles. The phoretic mites from the sampled beetles and those from the sediments were tallied separately.

All 232 specimens of the 12 species of mites collected in the study were examined for identifiable ascospores. No attempt was made to randomly sample the mites because many mite species were represented by only a single or just a few individuals, and because of the difficulties in counting and identifying spores on some of the mites. Each ascospore species on each specimen was counted and divided into two groups: those mites with < 30 of each ascospore species and those with > 30 (previous experience suggested that 30 spores was a valid number) (table 3). Seven types of ascospores were recognized. Ascospores of *Pyxidiophora* were identified as *Thaxteriola*-type species although conidia were not present. The Ophiostomataceae species were identified by the key in Solheim (1986). Most of the ascospores were readily recognizable; but in our unstained slide mounts of mites, the ascospore sheaths (and in particular the distinction between the sheaths and the spore walls) were difficult to see. This was especially true of the

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Table 1. *Mite species phoretic on adult* Ips typographus japonicus

Species	Number of mites
Trichouropoda polytricha (Vitzthum)	36
Trichouropoda hirsuta Hirschmann	1
Uroobovella ipidis (Vitzthum)	730
Uroobovella n.sp. no. 72	6
Dendrolaelaps quadrisetus (Berlese)	34
Total	150

hat-shaped ascospores resembling *Ophiostoma europhioides*. However, the 'small hat'-shaped spore was easily recognizable as of one of several yeast genera, probably *Hansenula* or *Pichia*. Various types of unidentifiable spores (US in table 3) probably yeasts, *Fusarium, Cladosporium, Graphium, Leptographium,* and *Trichoderma*, as well as various species of conidia, were also noted, but not counted.

Table 2. *Number of mites found in the vial sediments*

Species	No. of mites
Pleuronectocelaeno japonica Kinn	3
*Trichouropoda polytricha**	35
*Uroobovella ipidis**	12
*Dendrolaelaps quadrisetus**	1
Paraleius leontonychus (Berlese)	6
Calvolia n.sp. no. 33	
[1]New genus (near *Histiogaster*) n.sp. no. 27	1
Histiostoma piceae Scheucher)	1
Histiostoma vitzthumi Scheucher (= *H. serrata* Vitzthum)	1
Iponemus gaebleri (Schaarschmidt)	21

*These species were also present on the adult beetles (Table 1)
[1]'New genus (near *Histiogaster*) n. sp. no. 27' was recently identified by B. M. OCONNOR as '*Calvoliella* probably *stammeri* (TÜRK and TÜRK 1957).

3 Results and discussion

Of the 156 SBB, 30 beetles were sampled; 77% of the latter possessed at least one mite. The average number of mites per beetle was 5.0 for all sampled beetles, but 6.5 for those beetles with mites. This is a conservative estimate, because 82 mites fell off the beetles after they were placed in the alcohol.

Five mite species remained attached to the beetles (table 1). Of these, two species had not been recorded previously from *I. typographus. Uroobovella n.* sp. no. 72 is new to science and *Trichouropoda hirsuta* is noted in the Palearctic region for the first time. This latter species is frequently seen in galleries of bark beetles in North and Central America, and is commonly phoretic on the cerambycid, *Monochamus titillator* (F.) (KINN and LINIT, 1989; MOSER and ROTON, 1971). The number of phoretic mites attached to the 30 sample beetles numbered 150, which represented 65% of the 232 mites recovered. The numbers of mites per beetle ranged from 0 to 31. Mites per beetle from the sampled beetles totalled 6.5 for May and 8.0 for June, but only 0.5 for July. All 20 beetles from May and June had at least one mite per beetle; but of the 10 beetles sampled in July, seven were free of mites and the others had no more than two.

The 82 individuals found in the vial sediments (table 2) were fewer in number, but represented twice as many species than those attached to the beetles (table 1). In all, the sediment samples totalled 10 mite species comprising 35% of the 232 mite individuals tallied. Four of the seven species found in the vial sediments, but not attached to the beetles, were represented by only a single specimen.

A total of 12 species were found with both the sampled beetles and the vial sediments. The biologies of most are unknown, but *Iponemus gaebleri*, at least, is a known parasitoid, preying on the eggs of *Ips* spp. (GAEBLER, 1947). The two species of *Histiostoma* are probably filter feeders (OCONNOR, 1982); *Dendrolaelaps quadrisetus* and perhaps other mite species consume nematodes (KINN, 1967). Of the 10 beetles sampled in May, all contained large numbers of phoretic nematodes, which rode under the elytra.

In Japan, the average number of mites from all sampled beetles was 5.0, v.s. only 3.3 from Germany and 4.4 from Sweden. Moreover, the percentage of Japanese beetles possessing one or more mites was 77% — almost three times greater than those from Europe. In contrast, four times as many mite species were found in Europe. Perhaps some of these differences, as well as differences in numbers of mites reflect the smaller number of SBB collected and sampled from Japan.

Mite species common (> 9%) in Japan were also common in Europe; likewise, rare species (< 1.3%) in Japan were also rare in Europe. One exception was *Histiostoma piceae*, common in Europe, but with only one specimen from Japan. Three species (*Pleuronectocelaeno japonica, Trichouropoda hirsuta,* and *Uroobovella n.* sp. no. 72) were all uncommon in Japan, but did not occur in Europe. One of these, *P. japonica* (KINN, 1991), the largest mite collected, was described as new from this material. This species is especially interesting because a closely related species, *P. barbara*, was present in the SBB collections from Sweden (MOSER et al., 1989a). This mite is a highly variable and widely distributed species associated with *Ips* and *Pityogenes* attacking *Pinus* and *Picea* throughout Europe, North Africa, and North America (KINN, 1991). Thus, the geographical isolation of the Japanese *Pleuronectocelaeno* populations from those of the mainland seems to have resulted in classic speciation from the ancestral population. It will be interesting to see which of these two species occurs with the SBB populations of China and Korea.

Of the 12 mite species collected from Japan, all but two carried one or more spore types on their bodies (table 3). These two mite species were represented by only one specimen each; additional specimens would have likely revealed the presence of spores. Of the 232 mites examined, 83% carried a spore of some kind; but only 38% hosted one or more types of ascospores: 143 supported no ascospores; 69 carried one ascospore species; 15 two species, and five had three species. The five individuals carrying three ascospore species consisted of three *Trichouropoda polytricha*, one *Uroobovella n.*

Table 3. *Mite species examined and estimates of microorganism numbers*

Ascospore types* / Mite species	Mite #'s	THAX L	THAX H	BIC L	BIC H	EUR L	EUR H	MIN L	MIN H	PIC L	PIC H	POL L	POL H	Y HAT L	Y HAT H	US
		Number of mite individuals with spore types														
Pleuronectocelaeno japonica	3															2
Trichouropoda polytricha	71	—	—	15	—	1	—	2	—	1	14	1		9	2	57
T. hirsuta	1	—		1		—	—	—	—	—	—	—	—			1
Uroobovella ipidis	85	—	—	15	—	—	—	3	—	1	—	—	—	2	—	76
U. n. sp. no. 72	6	—	—	7	—	—	—	—	—	—	—	1	—	—	—	5
Dendrolaelaps quadrisetus	35	—	—	6	9	—	—	—	—	—	—	1	—	3	—	18
Paraleius leontonychus	6	1	—	2	—	—	—	—	—	—	—	—	—	—	—	4
Calvolia n.sp. no. 33	1	—	—	—	1	—	—	1	—	—	—	—	—	1	—	1
New genus (near *Histiogaster*) n. sp. no. 27	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
Histiostoma piceae	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H. vitzthumi	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iponemus gaebleri	21	—	—	10	—	—	—	—	—	—	—	1	—	4	—	14
TOTALS	232	1	—	56	10	1	—	6	—	3	14	4	—	19	2	179

*THAX, spores of *Pyxidiophora* spp. with pore in darkened holdfast region; BIC, *Ophiostoma bicolor* Davids. & Wells; EUR, *O. europhioides* (Wright & Cain) Solheim; MIN, *Ceratocystiopsis minuta* (Siem.) Upadhyay & Kendrick; PEN, *O. penicillatum* (Grosm.) Siem.; PIC, *O. piceae* (Münch) H. & P. Sydow; POL, *Ceratocystis polonicum* Siem.; Y/HAT, yeast "hat" probably *Hansenula* sp. and/or *Pichia* sp.; US, unidentified spores; L, less than 30 ascospores/specimen; H, more than 30 ascospores/specimen. Example: six individuals of *Dendrolaelaps quadrisetus* each contained less than 30 ascospores of *O. bicolor*; nine individuals had more than 30.

sp. no. 72, and one *Iponemus gaebleri*. All except the latter are relatively large mites.

The ascospores stuck anywhere on the mite bodies, with no special carrying structures (sporothecae) evident. In table 3, the numbers of ascospores carried by the mites may be underestimated, because it is possible that large numbers of spores were washed off in the alcohol storing the beetles, or lost in processing the mites for mounting on slides. Numbers of spores on individual mites varied from a single spore to hundreds, but most ascospore species were present on mites in numbers less than 30.

Ophiostoma bicolor was the most common ascospore type, occurring on 28% of the 232 mite specimens and on seven mite species. The next most common were the unidentified 'small hat'-shaped yeast spores on 9% of the specimens and on five species, followed by *O. piceae* on 7% of the specimens and on three mite species. But the latter may have had the most total ascospores because it was the most common ascospore species having more than 30 specimens per mite. *Ceratocystis polonicum*, the most pathogenic fungus, occurred on only 2% of the specimens and in small numbers on four mite species. WINGFIELD et al. (1995) may provide a reason for these low numbers. They suggest that fungal species with low virulence (such as *Ophiostoma* spp.) are strongly antagonistic toward the more virulent species (such as *Ceratocystis polonicum*).

The *Thaxteriola* (BLACKWELL et al., 1986) was only seen on one mite specimen. Unidentifiable spores (US) were present on 77% of the mite individuals, and were present on all the mite species except the two *Histiostoma* individuals.

With the exception of *Ophiostoma penicillatum*, all of the ascospore types found in Japan were also present in a similar mite survey for Sweden (MOSER et al., 1989b). But this species as well as *O. bicolor*, *O. europhioides*, *O. ainoae*, and *O. piceae* were isolated from trees killed by SBB in Japan (YAMAOKA and TAKAHASHI, 1995). The percentage of mite individuals carrying an ascospore of some kind was similar for Japan and Sweden, *O. bicolor* was the most frequent species, and no correlation between ascospore type and mite species was noted for either country.

4 Conclusions

This study illustrates that a great deal of information can result from the survey of relatively few bark beetles when time and labour are important constraints to research. Twelve species of mites and at least four species of fungi are recorded here for the first time for Japan. The latter includes the virulent tree disease *Ceratocystis polonicum*, at least, which is apparently capable of being transmitted by SBB mites. Future studies should determine if a paucity of mite species really exists in Japan (compared to that of Europe), or if more sampling is needed. Emphasis here should be placed on two important mite parasitoids, *Pyemotes dryas* (Vitzthum) and *Paracarophaenax ipidarius* (Redikortsev). If they are not native, then consideration should be given to introducing them as classic biocontrol agents for SBB. *P. dryas*, at least, has been located as far East as Gansu Provence, China, phoretic on *Polygraphus poligraphus* ex. *Picea asperata* (Moser, unpublished). At least one mite, *Pleuronectocelaeno japonica*, may have evolved in Japan; another, *Trichouropoda hirsuta*, is a North American species not yet recorded for the rest of Eurasia.

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