

Phoretic mites and nematode associates of *Scolytus multistriatus* and *Scolytus pygmaeus* (Coleoptera: Scolytidae) in Austria

John C. Moser, Heino Konrad*, Thomas Kirisits* and Lynn K. Carta†

USDA Forest Service, Southern Research Station, 2500 Shreveport Highway, Pineville, LA 71360, U.S.A., *Institute of Forest Entomology, Forest Pathology, and Forest Protection (IFFF), Department of Forest and Soil Sciences, BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Hasenauerstrasse 38, A-1190 Vienna, Austria and †USDA-ARS, Nematology Laboratory, Beltsville, MD 20705, U.S.A.

- Abstract**
- 1 The species assemblages and abundance of phoretic mites and nematodes associated with the elm bark beetles, *Scolytus multistriatus* and *Scolytus pygmaeus*, were studied in Austria.
 - 2 A total of 3922 individual mites were recorded from 144 adults of *S. multistriatus* and 178 adults of *S. pygmaeus*. The species spectrum was identical and the relative abundance of mites was very similar for both species of scolytids. Nine mite species, *Pyemotes scolyti*, *Pseudotarsonemoides eccoptogasteri*, *Trichouropoda bipilis*, *Tarsonemus crassus*, *Proctolaelaps eccoptogasteris*, *Proctolaelaps scolyti*, *Chelacheles michalskii*, nr. *Eueremaeus* sp. and *Elattoma* sp. were detected. Two of the nine species, nr. *Eueremaeus* sp. and *Elattoma* sp., are documented here as new associates of *Scolytus* spp.
 - 3 *Pyemotes scolyti* was the most frequent mite species, and *Ps. eccoptogasteri* and *T. bipilis* were relatively common, whereas the other mites occurred occasionally or were rare.
 - 4 The trophic roles of most of the mites associated with *S. multistriatus* and *S. pygmaeus* are poorly known, but they may include fungivores, parasitoids of bark beetle broods, predators of bark beetle broods and/or mites and/or nematodes.
 - 5 Besides phoretic mites, two nematode associates were seen on the investigated insects. A species of *Cryptaphelenchus* occurred under the elytra of both scolytid species, whereas the adults of a *Neoparasitylenchus* sp. were present inside abdomens of *S. multistriatus*, but absent from *S. pygmaeus*.

Keywords Dutch elm disease, elm, nematode, *Ophiostoma novo-ulmi*, phoresy, Scolytidae, mite, *Scolytus multistriatus*, *Scolytus pygmaeus*, *Ulmus minor*.

Introduction

Subsequent to the appearance of Dutch elm disease (DED) at the beginning of the 20th century, the elms (*Ulmus* spp.) in Europe, North America and parts of Asia have been seriously affected by this destructive vascular wilt disease that is caused by two closely related species of *Ophiostoma* (Ascomycota). Although the first wave of DED was caused by *Ophiostoma ulmi* (Buism.) Nannf., the second and still ongoing pandemic from the 1940s onwards has been triggered by the two subspecies (ssp. *americana* Brasier & Kirk

and ssp. *novo-ulmi* Brasier) of *Ophiostoma novo-ulmi* Brasier and hybrids between them (Brasier, 1991, 2000; Brasier & Kirk, 2001; Konrad *et al.*, 2002). *Ophiostoma ulmi* and *O. novo-ulmi* are amongst the best known examples of tree pathogens that are vectored by insects, in this particular case by elm bark beetles in the genus *Scolytus* Geoffroy and in North America also by *Hylurgopinus rufipes* (Eichhoff) (Lanier & Peacock, 1981; Webber & Brasier, 1984; Webber & Gibbs, 1989; Webber, 1990, 2000).

Scolytus multistriatus (Marsham) and *Scolytus pygmaeus* (Fabricius) are among the guild of eight *Scolytus* species infesting elm trees in Europe (Pfeffer, 1995). In the 20th century, *S. multistriatus* has also been introduced and established in North America (Lanier & Peacock, 1981), Australia (Lanier & Peacock, 1981) and New Zealand

Correspondence: John C. Moser. Tel.: +1 318 4737258; fax: +1 318 4737222; e-mail: jmoser@fs.fed.us

(Gadgil *et al.*, 2000). Both *Scolytus* species are regarded as secondary bark beetles that breed in stressed elm trees, elm logs and branches (Postner, 1974). The most important impact of *S. multistriatus* and *S. pygmaeus* is related to their role as vectors of the DED pathogens from diseased to healthy trees during maturation feeding on the bark of twigs and in twig crotches in the crown of healthy elm trees (Lanier & Peacock, 1981; Webber & Brasier, 1984; Webber & Gibbs, 1989; Webber, 1990, 2000; Basset *et al.*, 1992; Faccoli & Battisti, 1997). *Scolytus* species do not possess a mycelium and, instead, transmit ascospores and conidia of *Ophiostoma novo-ulmi* on their body surface (Lanier & Peacock, 1981; Webber & Brasier, 1984; Webber & Gibbs, 1989). Considering the importance of *Scolytus* spp. in the transmission of the DED pathogens, control measures against the insects form an essential part of integrated management strategies against Dutch elm disease (Campana & Stipes, 1981; Gadgil *et al.*, 2000).

Eight species of phoretic mites, *Chelacheles michalskii* Samsinak, *Histiostoma ulmi* Scheucher, *Pseudotarsonemoides eccoptogasteri* Vitzthum, *Proctolaelaps eccoptogasteris* (Vitzthum), *Pr. scolyti* Evans, *Pyemotes scolyti* (Oudemans), *Tarsonemus crassus* (Schaarschmidt), and *Trichouropoda bipilis* (Vitzthum) have been recorded in Europe phoretic on *S. multistriatus* (Marshall) and/or *S. pygmaeus* (Fabricius), or from *Scolytus* galleries on *Ulmus* spp. (Vitzthum, 1920, 1921, 1923; Oudemans, 1936; Scheucher, 1957; Evans, 1958; Schaarschmidt, 1959; Samsinak, 1962). Except for *Pr. scolyti* and *Py. scolyti*, a somewhat different guild of mite species may be associated with *S. multistriatus* in North America. Smiley & Moser (1974) described the following new taxa: *Heterotarsonemus milleri* Smiley & Moser, *Pseudotarsonemoides scolyti* Smiley & Moser, *Tarsonemus kennedyi* Smiley & Moser and *Ununguitarsonemus peacocki* Smiley & Moser, all of which were either phoretic on the insects or found in galleries of *S. multistriatus* on *Ulmus americana* L. To date, none of these four mites has been recorded in Europe where the insect is native.

Although a great deal of literature has been devoted to the bark beetles attacking *Ulmus* and the pathogenic fungi that these beetles transmit, the literature is scant and fragmented concerning the mites known to be carried by these scolytids. Except for *Py. scolyti*, little is known concerning the biologies of the above mentioned mites, except for brief accounts of their phoretic habits and/or gallery associations. Studies on the phoretic mites of conifer bark beetles have revealed that these arthropods have a number of trophic roles in the insect galleries, including beetle parasitoids, insect and nematode predators, fungivores, and omnivores (Kinn, 1983; Klepzig *et al.*, 2001a, 2001b). Perhaps the most intriguing role that mites play on conifers is related to the transmission of tree pathogens, mycelial symbionts and fungal antagonists of bark beetles (Bridges & Moser, 1983; Moser *et al.*, 1989b, 1995, 1997; Klepzig *et al.*, 2001a, 2001b). Although the mite taxa associated with scolytids attacking *Ulmus* spp. differ, their roles may be similar to the mite associates of conifer bark beetles. Previously, mites have been reported to enhance dissemination of conidia and ascospores of *O. ulmi* and *O. novo-ulmi*

during their saprotrophic phase in and around bark beetle galleries in the bark of elm trees, thereby promoting sexual reproduction and recombination (Brasier, 1978).

As an initial step in the study of the trophic relationships between *Scolytus* spp., phoretic mites, *O. novo-ulmi* and their elm hosts, we present the first comprehensive list of mites phoretic on *S. multistriatus* and *S. pygmaeus* attacking *Ulmus* spp. and provide data on their relative phoretic abundance on *S. multistriatus* and *S. pygmaeus*. In addition, we report on two nematode associates of the two *Scolytus* species found on elm in Austria.

Materials and methods

On 17 May 2002, five stem sections, each approximately 120 cm in length and 20 cm in diameter, were cut from a European field elm, *Ulmus minor* Mill., near Güssing, Burgenland, Austria, at 300 m a.s.l. This tree had been declining due to DED, and was subsequently attacked by *S. multistriatus* and *S. pygmaeus*. Originally, we also intended to include *Scolytus scolytus* Olivier for investigation of phoretic mites, but this scolytid had not infested the elm tree from which samples were collected. The stem sections were placed in a laboratory rearing cage at 20 ± 1 °C. Beetles emerged from 28 May to 30 June 2002, and flew to the cage screens where they were collected on 10 dates, and placed in vials containing 70% alcohol. The two beetle species were separated and each placed in separate vials, totalling 10 vials for each scolytid. The beetles were then transferred to lactophenol for clearing. Mites still attached to the beetles were tallied separately from those that fell off the beetles into the lactophenol, and those that separated from the beetles as they were placed in alcohol after being picked off the cage screen (Tables 1 and 2). Voucher specimens (slides) of all mite species detected in this study are maintained in the collections of the authors, and in other collections of mite taxonomists mentioned in the acknowledgements.

Nematodes were fixed within the insects in 70% alcohol, transferred to lactophenol for clearing, mounted in Berlese fluid (Humason, 1972) or glycerin, and measured with an ocular micrometer. Specimens were viewed with differential interference optics, images were captured with Image-Pro Plus™, version 4.1 (Silver Spring, Maryland), and images stitched together in Adobe Photo Shop, version 7.0 (Adobe Systems Inc., San Jose, California). Nematode slides and beetles with aphelenchid nematodes in alcohol were deposited in the USDA Nematode Collection, Nematology Laboratory, Beltsville, Maryland.

Results and discussion

Phoretic mites and nematodes from *Scolytus multistriatus* (Table 1)

A total of 144 individuals of *S. multistriatus*, consisting of 56 (39%) males and 88 (61%) females, were collected after emergence from the elm logs, and examined for mites and

Table 1 Summary of mites phoretic on 56 male and 88 female *Scolytus multistriatus*

Mites species	Phoretic stage	On Beetles	Lactophenol sediments	Alcohol sediments	Totals
<i>Chelacheles michalskii</i>	Female	0	2	29	31
<i>Elattoma</i> sp. ^a	Female	0	0	4	4
nr. <i>Eueremaeus</i> sp.	Female	0	0	1	1
<i>Proctolaelaps eccoptogasteris</i>	Female	0	0	1	1
<i>Proctolaelaps scolyti</i>	Female	2	0	2	4
<i>Pseudotarsonemoides eccoptogasteri</i>	Female	33	12	49	94
<i>Pyemotes scolyti</i>	Female	272	91	1067	1430
<i>Tarsonemus crassus</i>	Female	2	2	18	22
<i>Trichouropoda bipilis</i>	Deutonymph	71	19	3	93
Total		380	126	1174	1680

^aTotals from 59 beetles sampled.

nematodes. Twenty-two percent of the males and 25% of the females were devoid of mites, but these percentages are probably considerably inflated because of the large numbers of mites that fell off the insects, and were thus present in the alcohol and lactophenol sediments. Nine mite species, including *Py. scolyti* (Fig. 1b) *Ps. eccoptogasteri* (Fig. 1j), *T. bipilis* (Figs 1g and h), *T. crassus* (Fig. 1e), *Pr. scolyti* (Fig. 1c), *C. michalskii* (Fig. 1a), nr. *Eueremaeus* sp. (Fig. 1i), *Pr. eccoptogasteris* (Fig. 1d) and *Elattoma* sp. (Fig. 1f) were detected among the 1680 individuals examined (Table 1). The designation of nr. *Eueremaeus* sp. to the genus *Eueremaeus* is provisional, because the taxonomic status of this genus requires revision (R. A. Norton, personal communication).

Previous experience has shown that intensive sampling of a single tree, such as we performed in the present study, will reveal most or all of the commonly associated mite species, although many of the uncommon or rare associates may be missed (Moser & Roton, 1971a; Moser & Bogenschütz, 1984; Moser *et al.*, 1989a; Moser *et al.*, 1997).

Two of the nine mite species, nr. *Eueremaeus* sp. and *Elattoma* sp., are documented here as new associates of *S. multistriatus*. However, *H. ulmi*, which has previously been reported from *Scolytus* spp. on elm in Europe (Scheucher, 1957), was not detected. Approximately 85% of the 1680 mites belonged to *Py. scolyti*, which was thus by far the most common species. *Pseudotarsonemoides eccoptogasteri*

and *T. bipilis* were relatively common, whereas the other mites occurred occasionally (*Elattoma* sp., *T. crassus* and *C. michalskii*) or were rare (nr. *Eueremaeus* sp., *Pr. eccoptogasteris* and *Pr. scolyti*) (Table 1). Both nr. *Eueremaeus* sp. and *Pr. eccoptogasteris* were represented by only one individual in the sample of mites from *S. multistriatus*. Of the latter species, only two museum specimens have previously been known to exist (Evans, 1958) and the two females collected from *S. multistriatus* and *S. pygmaeus* (Table 2) in this study double the world supply of this mite species. *Tarsonemus crassus* appears to adhere weakly to the beetles because it was predominantly found in the alcohol sediments, and this probably explains why the number of voucher specimens of this mite in collections is low too (Magowski & Moser, 2003).

With the exception of *T. bipilis*, which occurred most frequently directly on the beetles, the majority of the individuals of all other mite species recovered from *S. multistriatus* were found in the alcohol sediments (Table 1). This suggests that the latter mites were phoretic on the body surface of the insects, and were not tightly attached to them or protected under the elytra. *Trichouropoda bipilis*, which tightly cements itself to the beetle body with an anal tube, was the only species whose numbers were higher on the beetles than in the sediments. All species other than the uropodid attached by claws to the beetle setae with various degrees of success, as indicated by the proportion of mites

Table 2 Summary of mites phoretic on 67 male and 111 female *Scolytus pygmaeus*

Mites species	On beetles	Lactophenol sediments	Alcohol sediments	Totals
<i>Chelacheles michalskii</i>	1	1	8	10
<i>Elattoma</i> sp. ^a	0	2	25	27
nr. <i>Eueremaeus</i> sp.	0	1	0	1
<i>Proctolaelaps eccoptogasteris</i>	0	0	1	1
<i>Proctolaelaps scolyti</i>	0	2	6	8
<i>Pseudotarsonemoides eccoptogasteri</i>	171	42	209	422
<i>Pyemotes scolyti</i>	501	243	958	1702
<i>Tarsonemus crassus</i>	0	4	20	24
<i>Trichouropoda bipilis</i>	31	12	4	47
Total	704	307	1231	2242

^aTotals from 52 beetles sampled.

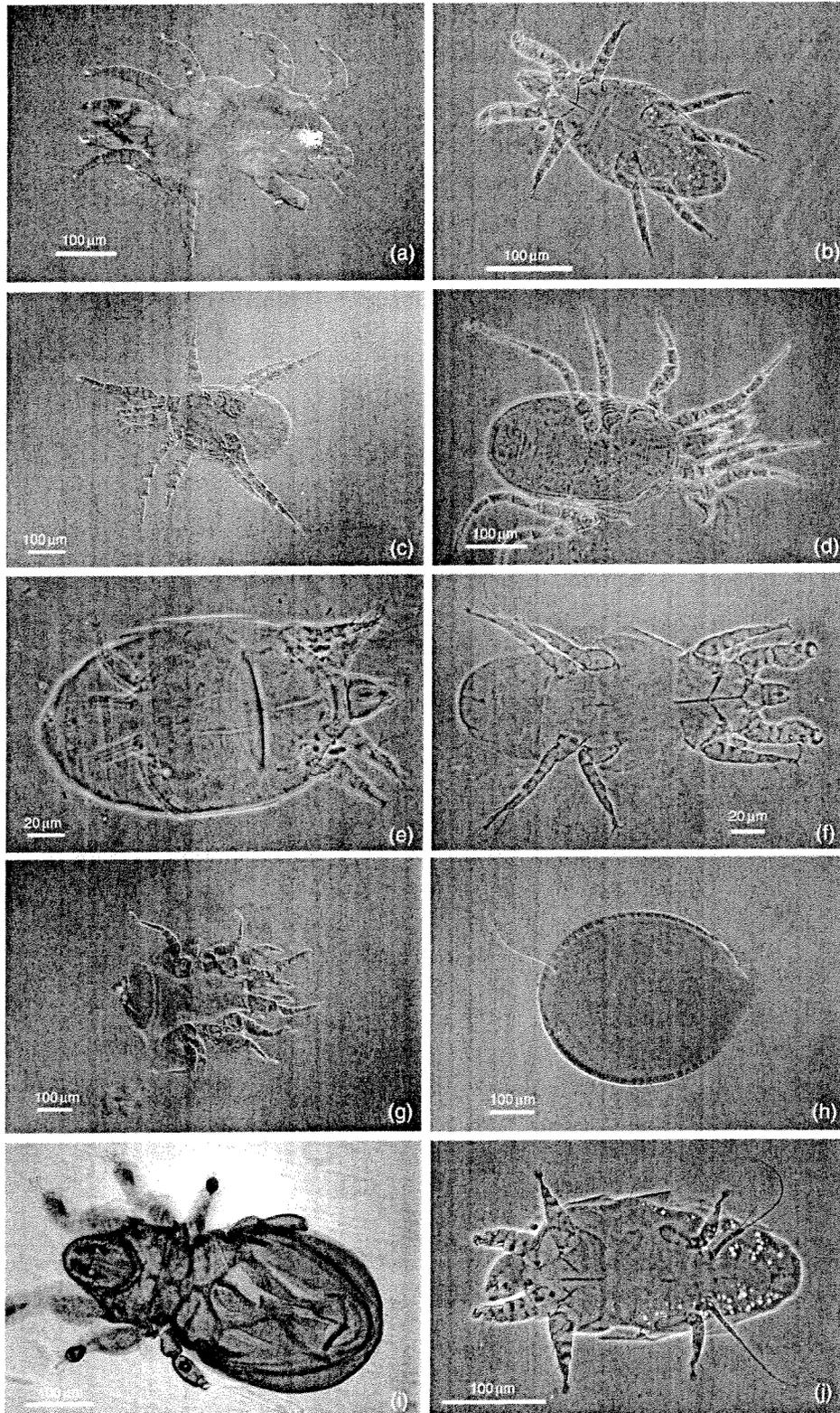


Figure 1 Phoretic mites from *Scolytus multistriatus* and *Scolytus pygmaeus* in Austria: (a) *Chelacheles michalskii*, (b) *Pyemotes scolyti*, (c) *Proctolaelaps scolyti*, (d) *Proctolaelaps eccoptogasteris*, (e) *Tarsonemus crassus*, (f) *Elattoma* sp., (g) *Trichouropoda bipilis* (ventral view), (h) *Trichouropoda bipilis* (dorsal view), (i) nr. *Everemaeus* sp., (j) *Pseudotarsonemoides eccoptogasteri*.

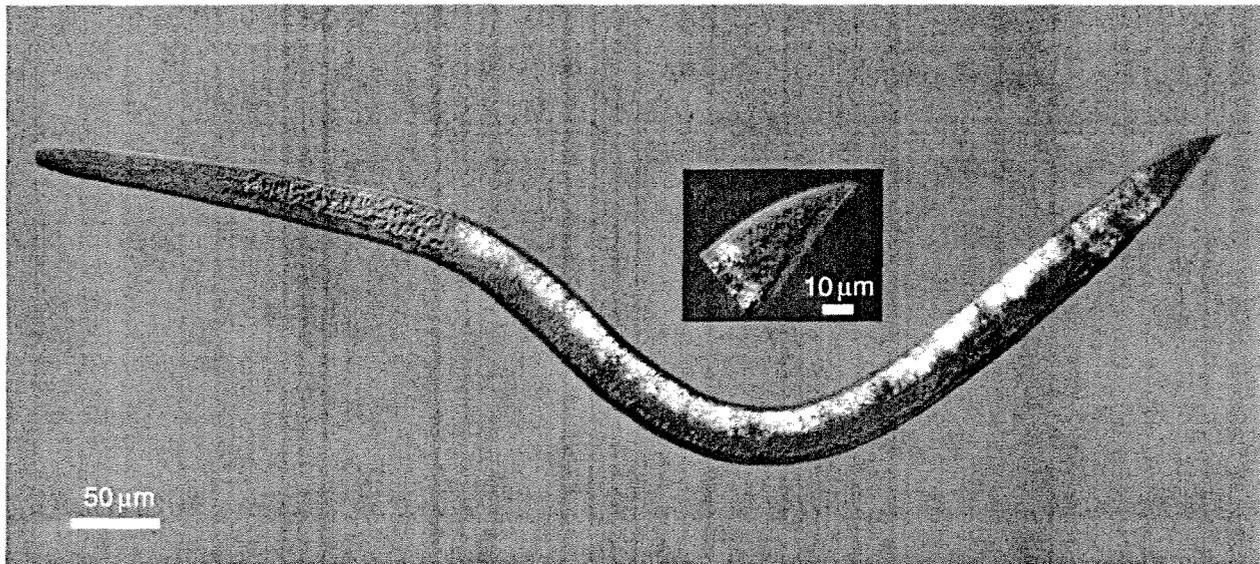


Figure 2 Nematode associate of *Scolytus multistriatus* and *Scolytus pygmaeus* in Austria: adult of *Neoparasitylenchus* sp.

detected directly on the beetles, as well as in the lactophenol and alcohol sediments (Table 1).

The preferred location of attachment to the insects differed by mite species. Although *T. bipilis* occupied a ventral position on the abdomen, *Py. scolyti* and *Ps. eccoptogasteri* preferred to attach on coxae 1 and 2. Even though the attachment site for *Elattoma* sp. was not seen, this species may prefer clinging to legs 1 and 2, similar to the preferences of *E. bennetti* (Cross & Moser) (Cross & Moser, 1971). With their enormous leg 1 claws that are modified for attachment to beetles, the phoretomorph stages of *Py. scolyti*, *Ps. eccoptogasteri*, and *Elattoma* sp. are representative for mite species that have apparently lost their normal

stages and have become highly adapted for phoresy on bark beetles and other insects (Moser & Cross, 1975). For the mite species that were not at all or rarely seen on the beetles, it is unclear where they preferentially attach to the insects. It is worthy of note that few mites were carried under the beetle elytra. This is in contrast to conifer bark beetles, where subelytral mites are common (Moser & Roton, 1971a). A few individuals of *Py. scolyti* and *Ps. eccoptogasteri* were seen in this location, but only when their preferred points of attachment were occupied.

Abdomens of 66% of the males and 88% of the females of *S. multistriatus* were infested by numerous individuals of a large, adult nematode. This 720–750- μm long nematode

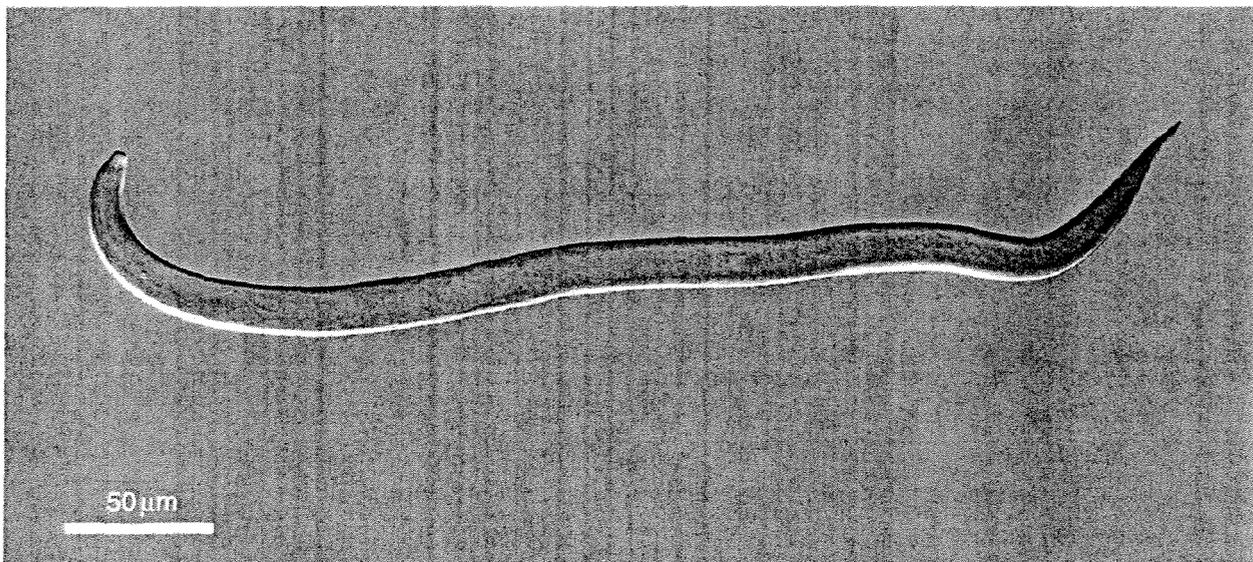


Figure 3 Nematode associate of *Scolytus multistriatus* and *Scolytus pygmaeus* in Austria: female of *Cryptaphelenchus* sp. (order Tylenchida, suborder Aphelenchina).

was identified as a *Neoparasitylenchus* species (Fig. 2), similar to *Neoparasitylenchus scolyti* (Oldham) (Nickle, 1967) (= *Parasitylenchus scolyti*; Oldham, 1930) that occurred in the body cavity near reproductive organs of *S. scolytus* and was reported to cause sterility in both sexes of this scolytid species (Oldham, 1930; Laidlaw, 1932). Oldham (1930) and Laidlaw (1932) reported that 60% of the beetle population was infested by *N. scolyti*, and 40% of the insects were classified as sterile. Although the *Neoparasitylenchus* sp. found in the present study was present in a similar position in *S. multistriatus* as reported for *N. scolyti* in *S. scolytus* (Oldham, 1930; Laidlaw, 1932), none of the abdominal organs appeared to be harmed. Under the elytra of both *S. multistriatus* and *S. pygmaeus*, we also observed smaller female nematodes (285–350 µm long) belonging to the genus *Cryptaphelenchus* (Fig. 3) (order Tylenchida, suborder Aphelenchina) (Hunt, 1993). Morphological characters and morphometric measurements did not correspond to species described from various scolytids in Germany (Fuchs, 1930; Fuchs, 1937; Rühm, 1956; Wegensteiner & Weiser, 1996). Accurate numbers of infested beetles were not determined, but this small aphelenchid nematode species generally appeared to be more abundant on *S. pygmaeus* than on *S. multistriatus*. Oldham (1930) originally reported adults of *N. scolyti* to be associated with a juvenile tylenchid nematode, and assumed that both nematodes belonged to the same species. However, later observations of beetles infested with the juvenile nematode, but not with adults of the parasitic *N. scolyti*, made this association suspect, and the juvenile nematode was later identified as a different taxon, *Parasitaphelenchus oldhami* Rühm (Rühm, 1956), with adults described much later (Hunt & Hague, 1974). This small *Cryptaphelenchus* species from *S. multistriatus* and *S. pygmaeus* in Austria differs from *P. oldhami* found in *S. scolytus* in Britain (Oldham, 1930; Rühm, 1956; Hunt & Hague, 1974) with respect to a shorter body, a short knobbed stylet, a prominent esophageal valve and a more attenuated tail. By contrast to the superficial position of the aphelenchid in the beetles from Austria, *P. oldhami* was found in the insect haemocoel. Neither *Cryptaphelenchus* spp., nor *P. oldhami* have been reported in *S. multistriatus* from the U.S.A., but aphelenchids, including an *Aphelenchoides* sp. (*parietinus* Bastian group), *Bursaphelenchus scolyti* Massey, and *Laimaphenenchus penardi* (Steiner) Fiipjev and Schuurmans Stekhoven (Massey, 1974) have been documented from *S. multistriatus* in the U.S.A. A third nematode, the free-living *Goodeyus ulmi* (Goodey, 1930) (Chitwood, 1933) (= *Cylindrogaster ulmi* Goodey, 1930), was found under the elytra of *S. scolytus* in Britain in association with *N. scolyti* (Oldham, 1930; Laidlaw, 1932), but this species was not observed in our study.

Phoretic mites and nematodes from *S. pygmaeus* (Table 2)

A total of 178 individuals of *S. pygmaeus*, consisting of 67 (38%) males and 111 (62%) females, were picked up after emergence from the elm logs, and examined for mites and

nematodes. The sex ratio for *S. pygmaeus* was virtually the same as that for *S. multistriatus*. Eighty-five percent of both *S. pygmaeus* sexes possessed at least one mite, which is worthy of note because this percentage is approximately 10% higher than that for the much larger *S. multistriatus*. In total, 2242 mites were collected from *S. pygmaeus*. The species spectrum of phoretic mites associated with *S. pygmaeus* was identical to that of *S. multistriatus* (Table 2). Similarly, the relative abundance of the various mite species and the proportions of individuals of each mite species recorded directly on the beetles, as well as in the lactophenol and alcohol sediments, were very similar for both scolytid species on elm (Table 2), indicating no substantial differences between them in terms of their mite associates. As on *S. multistriatus*, *Py. scolyti* was by far the most dominant mite phoretic on *S. pygmaeus*, accounting for 76% of the mite individuals detected. The most obvious difference between the mite associates of *S. multistriatus* and *S. pygmaeus* refers to *Ps. eccoptogasteri*, which was much more common on *S. pygmaeus*. In addition, *T. bipilis* and *C. michalskii* were slightly less common and *Elattoma* sp. was slightly more common on *S. pygmaeus* than on *S. multistriatus*. Numbers of the remaining mite species were approximately the same for both bark beetle species.

The major difference between the associates of *S. multistriatus* and *S. pygmaeus* refers to the nematodes because no adult nematodes representing the *Neoparasitylenchus* sp. from *S. multistriatus* were seen in the abdomens of *S. pygmaeus*. The same nematode occurring as females under the elytra of *S. multistriatus*, and representing a *Cryptaphelenchus* species, was also present under the elytra of *S. pygmaeus*. Although the *Cryptaphelenchus* sp. appeared to be more abundant on *S. pygmaeus* than under the elytra of *S. multistriatus*, visualization and counting of this nematode was difficult due to the same reason as that experienced for *S. multistriatus*.

Trophic roles of the mites associated with *S. multistriatus* and *S. pygmaeus*

The trophic roles of most of the mites associated with *S. multistriatus* and *S. pygmaeus* are poorly known but, from other bark beetle-mite-fungal systems, there is sufficient evidence to assume that their biology and ecology is diverse (Lombardero *et al.*, 2003; Moser *et al.*, 1995; Klepzig *et al.*, 2001b).

Kinn (1983) suggested that species in the genus *Proctolaelaps* may prey on nematodes. We observed a female and a larva of *Pr. scolyti* inside the abdomen of a single female of *S. multistriatus*. In Italy, Russo (1938) observed that *Pr. eccoptogasteris* nearly completely destroyed the eggs and larvae in galleries of *Phloeotribus scarabaeoides* (Bern.). *Pyemotes scolyti* is a common and well-known parasitoid of *Scolytus* spp. (Beaver, 1967; Krczal, 1959; Moser & Roton, 1971b). Although there are no field records of immature stages of *S. multistriatus* being attacked, the impact of this mite species may be substantial,

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