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Comparative analysis of the phoretic mites communities (Acari: Mesostigmata) associated with *Ips typographus* from natural and planted Norway spruce stands – Romania

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Abstract

A total of 1421 specimens of Ips typographus L. were examined for the presence of phoretic mites that were collected from twelve natural and planted Norway spruce forests in South-Western Romania. Seven phoretic mites were identified: Pleuronectocelaeno austriaca, Proctolaelaps fiseri, Vulgarogamasus oudemansi, Dendrolaelaps quadrisetus, Trichouropoda polytricha, Trichouropoda orszaghi and Uroobovella ipidis. The comparative analyses of the phoretic mite communities from natural and planted Norway spruce stands showed that there are significant differences in the species composition of the populations and their dominance classes but no significant differences in species diversity and numerical abundance.

Keywords: bark beetle, Mesostigmata, mites, natural forest, planted forest.

1. Introduction

The Carpathian forests, as well as all natural ecosystems, provide a full spectrum of goods and services that contribute to the socio-economic development of forest dependent human communities. Forest ecosystems are an important source of biological resources, regulating carbon sequestration, recycling nutrients and providing habitat services for species, as well as cultural services such as recreation. All these services are provided when the ecosystems are not disturbed by perturbations such as pollution, climate change, diseases (BYTNEROWICZ & al. [1]; DONIŢĂ and BIRIŞ [2]; PĂTRU –STUPARIU & al. [3]; WALENTOWSKI & al. [4]).

The Carpathian forests, which are dominated by Norway spruce, have suffered continual damage due to pests such as spruce bark beetles. The spruce bark beetle, *Ips typographus* L. is one of the main pest species of coniferous forests throughout Europe causing significant economic losses (HEDGREN [5]; FACCOLI and STERGULC [6]; KRŠIAK & al. [7]; CIESLA [8]; KRASCSENITSOVÁ & al. [9]).

Due to the huge impact of bark beetles on the health and economics of forest ecosystems, the ecology of their natural enemies has been the subject of many studies throughout Europe (MOSER and BOGENSCHUTZ [10]; MOSER & al. [11], [12]; BURJANADZE & al. [13];

TAKOV & al. [14]; GWIAZDOWICZ & al. [15], [16], [17]; KNEE & al. [18]; PENTTINEN & al. [19]; ČEJKA and HOLUŠA [20]).

During the last decade 274,444 ha of the 3 million hectares of the State forests in Romania have been affected by conifer bark beetles annually; 27% seriously (SIMIONESCU & al. [21]). The disasters generated by the very large explosions in the beetle populations have lead to the development of special research programmes for integrated biological control, based on autoecological studies of the beetles. These studies have revealed that *Ips typographus* L. is one of the most harmful pests of Norway spruce stands in Romania (MIHALCIUC and OLENICI [22]; OLENICI & al. [23]; DONIȚĂ and BIRIŞ [2]; DUDUMAN & al. [24]; SIMIONESCU & al. [21]; MANEA & al. [25]).

A major omission in these investigations has been the lack of studies of the diversity of phoretic mites living in the bodies of bark beetles or their galleries in the trees, in the Romanian forests. The only research that has been carried out was in the spruce forests of Braşov County (PARASCHIV [26]). New information about phoretic mites is important because several specialists in forestry management and forest pathology have affirmed that the mites could be a useful biological control tool for bark beetles, the Acari being able to feed on their eggs and larvae (KIELCZEWSKI and MICHALSKI [27]; GWIAZDOWICZ & al. [15]; PENTTINEN & al. [19]).

The main aims of this study were to:

1. determine the species diversity of the phoretic mites collected from *Ips typographus*;
2. undertake a comparative analysis of the phoretic mite communities from natural and planted Norway spruce stands from Romania.

2. Material and Methods

Twelve spruce stands were investigated, six located in the Ţarcu Mountains between 800–1400 m a. s. l. (Oţelul Roşu forest district- OR) and six sites in the Poiana Ruscă Mountains between 600–900 m a. s. l (Rusca Montană forest district- RM), from Caraş – Şeverin county, South-Western Romania (Figure 1).

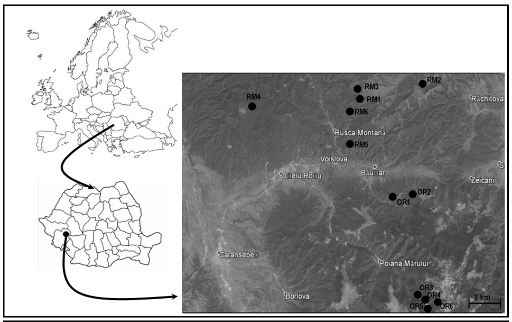


Figure 1. Geographical characterization of investigated natural (OR) and planted (RM) Norway forest stands from Caraş –Severin county, Romania

The study area includes 70-120 year old Norway spruce stands, plantation (RM) or natural forests (OR) (Table 1). The size of each investigated plots was by 1500 sq.m. For beetle collecting twelve barrier pheromone AtraTYP PLUS traps were used (six for each type of natural and planted forests). The traps, which were fabricated by the Institute of Chemistry, “Raluca Râpan” (Cluj-Napoca, Romania), comprised two plastic panels 60 cm x 40 cm, which were joined together. A hole by 10 cm x 15 cm was made about half way along the panels to attach the dispenser. This type of trap is provided with two funnels, one at the top and one under the panels. The second funnel has a plastic collector and a sieve. The total dimensions of the completed traps were 90cm x 40cm x 40cm. The traps were positioned 20 cm-30 cm from the edge of the forest close to areas with dead trees (Figure 2).

Beetle captures were collected every week from 18th April to 30th June 2013. After the numbers of adult beetles were counted, the living individuals were preserved in 90% alcohol for further analyses.

The mites were collected from the body surface, under elytra and from elytra declivity. The collected mites were placed in 70% ethanol, mounted on permanent or semi-permanent slides (using Hoyer’s medium or lactic acid, respectively), identified using the latest taxonomical literature and counted. The sample alcohol with biological material, which was not bonded by bark beetles, accidentally fallen of the *Ips typographus* was declared as sediment.

A B C

Figure 2. Collection methods for *Ips typographus* (A- barrier pheromone AtraTYP PLUS traps; B – sample with bark beetles; C - pheromone AtraTYP PLUS).

The statistical analysis was performed with PAST software (± = standard deviation SD; significance level= p) (HAMMER & al. [28]). Dominance classes (D %) were used as follows: eudominants (>30%); dominants (15.01–30%); subdominants (7.01–15.0%); residents (3.01–7%) and sub-residents (<3%) (BŁOSZYK [29]; GWIAZDOWICZ & al. [16], [17]).

Table 1. Description of the sampling sites

Site	Age (years)	Species composition	Type of forest	Treatment	Latitude/ longitude	Altitude (meters)	Exposure
RM 1	100	4S 4B 2Oh	Planted	Hygienic cuttings	N:45^{0}35’08’’ E:22^{0}29’29”	600	North
RM 2	80	6S 3B 1Oh	Planted	Hygienic cuttings	N:45^{0}36’56” E:22^{0}28’13”	700	North
RM 3	80	4S 1F 3B1Oh	Planted	Hygienic cuttings	N:45^{0}36’41” E:22^{0}30’26”	800	South
RM 4	90	5S 1F 4B	Planted	Hygienic cuttings	N:45^{0}35’49” E:22^{0}17’52”	900	North
RM5	95	5S 2F 3B	Planted	Hygienic cuttings	N:45^{0}36’70” E:22^{0}29’01”	700	North
RM6	100	6S 3F 1B	Planted	Hygienic cuttings	N:45^{0}36’11” E:22^{0}30’42”	700	South
OR1	100	8S 2B	Natural	Hygienic cuttings	N:45^{0}21’97” E:22^{0}34’22”	1000	North
OR2	110	6S 1F 3B	Natural	Clear cuttings in strips	N:45^{0}20’84” E:22^{0}36’41”	1100	South
OR3	90	9S 1Oh	Natural	Hygienic cuttings	N:45^{0}21’27” E:22^{0}36’60”	1100	South
OR4	75	8S 1Oh 1Lt	Natural	Thinning	N:45^{0}21’10” E:22^{0}37’21”	1200	South
OR5	100	8S 2Lt	Natural	Hygienic cuttings	N:45^{0}20’52” E:22^{0}37’41”	1300	North
OR6	80	8S 2Lt	Natural	Hygienic cuttings	N:45^{0}20’57” E:22^{0}38’36”	1400	North

S- Spruce; B- Beech; F- Fir; Oh- Other hardwoods; Lt- Locust tree

3. Results and Discussions

In total 1421 individuals of *Ips typographus* were captured and examined of these 42.08% had been colonized by mites. In the natural spruce stands, 666 individuals bark beetle were identified, with a mean of 111 ± 28.08 individuals per site of which only 41.44 % had been colonized by mites (Fig. 3). The mean number of *Ips typographus* per site that contained mites was 46 ± 14.11. In the planted forests, 755 individual bark beetles were found (with a mean number of 125.83 ± 36.71 per site); 42.64% were colonized by mites, with a mean value of 53.66 ± 21.82 per site (Figure 4).

The differences between the two types of forest are not statistically significant, either in the total number of identified bark beetles (p= 0.441) or in the total number of individuals colonised by mites (p = 0.632).

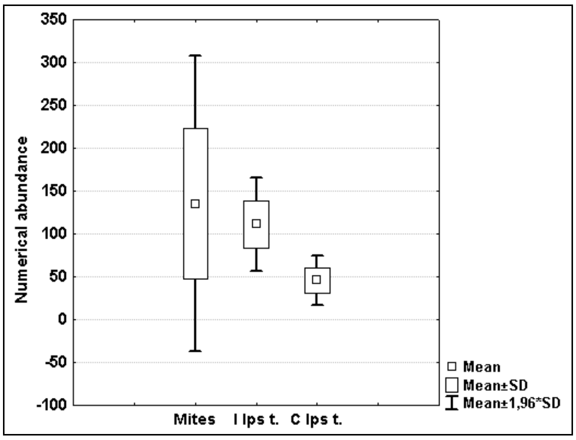


Figure 3. Mean values of numerical abundance of *Ips typographus* and Mesostigmata mites from natural (OR) forest Norway stands (I Ips t.= identified *Ips typographus*; C Ips t. = colonized *Ips typographus*).

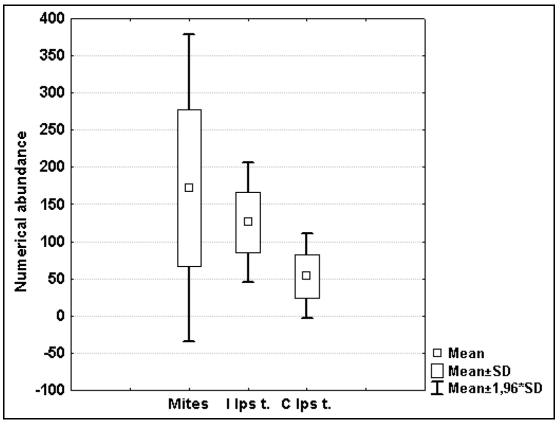
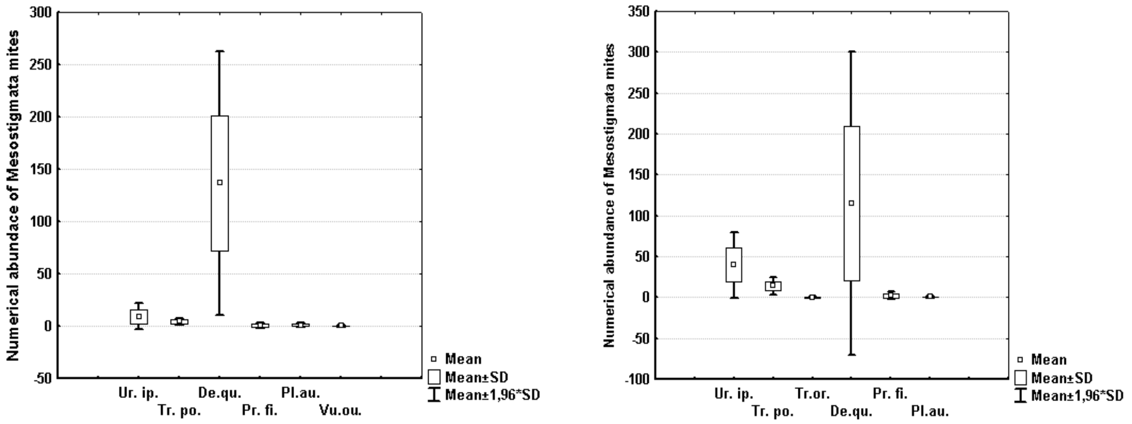


Figure 4. Mean values of numerical abundance of *Ips typographus* and Mesostigmata mites from planted (RM) forest Norway stands (I Ips t. = identified *Ips typographus*; C Ips t. = colonized *Ips typographus*).

If the Mesostigmata mite fauna is considered, seven species were identified, belonging to the six families: *Pleuronectocelaeno austriaca* (Celanopsidae), *Proctolaelaps fiseri* (Ascidae), *Vulgarogamasus oudemansi* (Parasitidae), *Dendrolaelaps quadrisetus* (Digamasellidae), *Trichouropoda polytricha, Trichouropoda orszaghi* (Trematuridae) and

Uroobovella ipidis (Urodinychidae). In the natural forests, six Mesostigmata mite species were identified, with a total abundance of 811 individuals (mean per site 135 ± 47.23). In the planted stands, the number of investigated mite species was similar but the numerical abundance was higher, by 1033 individuals (mean per site 172.16 ± 65.45). The difference between the numerical abundance of mite species in the natural and planted Norway spruce stands is not significant ($p = 0.814$) (Figures 3, 4). However when the mean number of mites per colonized bark beetles is taken into account the value is much higher in natural forest (0.74 ± 0.40) when compared to the planted forests (0.35 ± 0.12).

Although the species diversity is similar in natural and planted spruce stands (Shannon index = 1.47), the community structure is different. *Vulgarogamasus oudemansi* was found only in the natural spruce stands, and *Trichouropoda orszaghi* only in planted forests. Both species were founded in samples sediment. The affiliation of the species to the dominance classes also differs between the two forest stand types. On the one hand *Dendrolaelaps quadrisetus* is eudominant in both types of forests (with a mean number of individuals of 136.66 ± 64.44 in the natural areas and 115.16 ± 94.50 in the planted areas) (Figures 5 A, B). On the other hand, there are no dominant or sub-dominant species in the natural stands, only residents and sub-residents (Table 2). In the planted stands, *Uroobovella ipidis* and *Trichouropoda polytricha* were found to be dominant and sub-dominant respectively (Table 2). The mean numerical abundance of *Uroobovella ipidis* was 39.66 ± 20.61 whilst that of *Trichouropoda polyricha* was 14 ± 5.76 (Figure 5B).



A B

Figure 5. The numerical abundances of Mesostigmatid mites from natural (A) and planted (B) stands (Ur.ip. = *Uroobovella ipidis*; Tr. po. = *Trichouropoda polytricha*; Tr. or. = *Trichouropoda orszaghi* ; De. qu. = *Dendrolaelaps quadrisetus*; Pr. fi. = *Proctolaelaps fiseri*; Pl.au. = *Pleuronectocelaeno austriaca; Vu.ou. = Vulgarogamasus oudemansi*)

When the development stages (Ds) were considered it was found that the majority of the mites were deutonymphs (DN), with two exceptions: *Proctolaelaps fiseri* and *Pleuronectocelaeno austriaca*, which were found as adults, mainly females (Table 2). Both species were discovered in the sample sediment while the rests were removed from different parts of the beetles including the leg coxa, elytra, elytra declivity or abdomen. Two deutonymphs of *Trichouropoda orszaghi* were also identified in samples sediment.

In natural Norway spruce stands the number of bark beetles collected and the number that had been colonized by mites were lower than those in the planted forests. However, the statistical analyses demonstrated that the difference was not significant. The natural forest is a complex, stable ecosystem that is controlled by an important feature, auto-regulation. If the natural ecosystem is subject to a perturbation that does not exceed a critical level, it has the capacity to 'protect' itself. Studies of forest ecosystems have shown that long-term elevated levels of atmospheric nitrogen, sulphur depositions and elevated ozone levels could predispose trees to insect attacks and other stresses (BYTNEROWICZ & al. [1]; DREVER & al. [30]). This could be a possible explanation for the lower rate of attacks of bark beetles on the natural stands of Norway spruce than on the planted stands.

The situation is similar in relation to mite communities. The same number of species was recorded in both types of spruce stand; the difference in the numerical abundance between the natural and planted spruce forests was found not to be significant.

The results of this study are in accordance with those obtained by Polish researchers who found that the mean number of species, the mean abundance and the Shannon, and Evenness indices were not significantly different in natural and managed spruce forests (GWIAZDOWICZ & al. [16]).

Table 2. Species of mite (Acari: Mesostigmata) from natural and planted stands (A = numerical abundance; D = dominance; Ds= development stages)

Species	Natural stands (OR)			Planted stands (RM)		
	A (ind.)	D (%)	Ds (%)	A (ind.)	D (%)	Ds (%)
Uroobovella ipidis (Vitzhum, 1923)	54	6.66	100 DN	238	23.04	100 DN
Trichouropoda polytricha (Vitzhum, 1923)	25	3.08	100 DN	84	8.13	100 DN
Dendrolaelaps quadrisetus (Berlese, 1920)	723	89.15	100 DN	691	66.89	100 DN
Proctolaelaps fiseri (Samsinak, 1960)	3	0.37	100 ♀	14	1.36	14,28 ♂ + 85,72 ♀
Pleuronectocelaeno austriaca Vitzhum, 1926	5	0.62	20 ♂ + 80 ♀	4	0.39	25 ♂ +75 ♀
Vulgarogamsus oudemansi (Berlese, 1904)	1	0.12	100 DN			
Trichouropoda orszaghi Masan, 1999				2	0.19	100 DN
Total	**811**	**100**		**1033**	**100**	

In the twelve spruce stands investigated in this study seven species of Mesostigmata mites were identified. The dominant species, in both natural and planted forests, was *Dendrolaelaps quadrisetus,* which is the most common phoretic mesostigmatid mite in the spruce forests of Europe. *Dendrolaelaps quadrisetus,* is frequently accompanied by *Trichouropoda polytricha* and *Uroobovella ipidis,* both species have also been found in studies of mites in coniferous forest ecosystems in Georgia, Germany, Poland, Bulgaria, Finland, Czech Republic, Russia, Sweden (BURJANADZE & al. [13]; TAKOV & al. [14]; GWIAZDOWICZ & al. [17]; PENTTINEN & al. [19]; ČEJKA and HOLUŠA [20]). Research has shown that *Dendrolaelaps quadrisetus* is present in the imago of *Ips typographus* and the larval galleries of main, sister and second generations and that bark beetle eggs are eaten by this mite (MASLOV [31]; PENTTINEN & al. [19]).

Three other species, *Pleunonectocelaeno austriaca, Proctolaelaps fiseri* and *Vulgarogamasus oudemansi* were identified as being resident or sub-resident species. These phoretic species have also been reported to occur in small numbers in other European countries (MOSER & al. [11]; BURJANADZE & al. [13]; TAKOV & al. [14]; GWIAZDOWICZ & al. [15], [16], [17]; PENTTINEN & al. [19]; ČEJKA and HOLUŠA [20]). The lignicole species *Trichouropoda orszaghi* was found accidentally in the sample sediment (MASAN and SVATON, [32]).

The phoresy phenomen of Mesostigmata mites is highlighted by the dominance of individuals that are in their early developmental stages. Mites in the sub-order Uropodina (*Trichouropoda polytricha*, *Uroobovella ipidis*) and those in the sub-order Gamasina

(*Vulgarogamasus oudemansi*, *Dendrolaelaps quadrisetu*s) were only found as deutonymphs. In 2011, GWIAZDOWICZ & al. [15] stated that the large number of phoretic mites found on bark beetles is an efficient phoresy method used by mesostigmatid mites.

Only the adult stages of *Proctolaelaps fiseri* (females) and *Pleuronectocelaeno austriaca* (males and females) were recorded. This phenomenon has also been observed in the Czech Republic (ČEJKA and HOLUŠA [20]).

The low numerical abundance of mites may be due to the study being carried out during the first mating flight of the beetles. The young adults of the first generation spend a longer time within the bark galleries compared with the adults of the second generation. The consequence of this is likely to be that a lower number of mites were transported by *Ips typographus* during the research period.

In conclusion, seven Mesostigmata mite species were identified in natural and planted Norway spruce stands in Romania. The dominant species was *Dendrolaelaps quadrisetus* accompanied in the planted forest stands by *Trichouropoda polytricha* and *Uroobovella ipidis*. The comparative analysis of phoretic mite communities from natural and planted Norway spruce stands in Romania revealed that on the one hand there are no significant differences in species diversity and numerical abundance but on the other hand there are differences in the species composition and the affiliation of species to the different dominance classes.

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