ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 59 (1), 2007: 87-96

Study of Bark Beetle (Coleoptera, Scolytidae) Pathogens from Coniferous stands in Bulgaria

***Danail Takov*[1*], *Danail Doychev*[2], *Rudolf Wegensteiner*[3], *Daniela Pilarska*[1*]**

[1]Institute of Zoology, Bulgarian Academy of Sciences, 1, Tsar Osvoboditel Blvd., Sofia 1000, Bulgaria, e-mails: dtakov@yahoo.com, dpilarska@zoology.bas.bg; [2]University of Forestry, 10, Kl. Ohridski Blvd., Sofia 1756, Bulgaria, e-mail: doychev@abv.bg
[3]Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU, Hasenauerstraße 38, A-1190 Vienna, Austria, e-mail: rudolf.wegensteiner@boku.ac.at

Abstract: The pathogen species composition and distribution in a variety of bark beetle species from different coniferous stands was investigated. Seven pathogen species were recovered: *I. typographus* Entomopoxvirus (*It*EPV), *Malamoeba scolyti, Gregarina typographi*, *Menzbieria chalcographi*, *Chytridiopsis typographi*, *Nosema*-like microsporidium and *Beauveria bassiana. Nosema*–like microsporidium, *M. chalcographi* and *M. scolyti* are new reports for the Bulgarian fauna. *I. sexdentatus, Pityophthorus pityographus*, *Cryphalus saltuarius, Orthotomicus proximus* and *Polygraphus subopacus* are newly reported hosts for *B. bassiana, M. scolyti, Chytridiopsis cf. typographi* and *Gregarina cf. typographi* respectively. *G. typographi* appears to have the broadest host range the beetles we examined, followed by *C. typographi, M. chalcographi. M. scolyti* and *Nosema*-like microsporidium appear to have narrower host ranges.

Key words: Bulgaria, bark beetles, pathogens, infections, *Ips typographus, I. sexdentatus I. acuminatus, Pytiogenes chalcographus, Orthotomicus proximus, Polygraphus subopacus, Pityophthorus pityographus, Cryphalus saltuarius, Hylurgus ligniperda*

Introduction

Bark beetles are some of the most serious insect pests of coniferous trees in Bulgaria and throughout the European subcontinent. These pests cause enormous economic loss and during intensive outbreaks can attack healthy standing trees (TZANKOV, MIRCHEV 1985, GEORGIEV 2006, ROSSNEV *et al.*).

Control of bark beetles is very difficult because of their cryptic habits: the majority of the life cycle stages occur beneath the bark of host trees. The most commonly used controls against these pests are sanitation measures, but these are expensive and very often lead to large clear-cut areas. Biological control with the use of natural enemies such as pathogens could be very helpful, if the pathogens could be manipulated in augmentative releases to initiate epizootics.

Pathogens of *I. typographus* have been intensively studied (WEISER 1954, 1955, 1977, WEGENSTEINER 1994, WEGENSTEINER, WEISER 1995, 1996, WEGENSTEINER *et al.* 1996, WEISER *et al.* 1997, 2003, ZIZKA *et al.* 1997, HÄNDEL *et al.* 2003, WEGENSTEINER, WEISER 2004), but there are few publications concerning pathogens of other bark beetles species, which attack coniferous trees (PURRINI 1980, PURRINI, WEISER 1985, KOHLMAYER *et al.* 2003, HÄNDEL *et al.* 2003). In Bulgaria there

*Corresponding authors

is only one published report on pathogens of *I. typographus* collected from the Vitosha and the Rhodope mountains (Takov *et al.* 2006).

The aim of this study is to investigate the pathogen species composition and distribution in a variety of bark beetle species from different coniferous stands.

Material and Methods

Bark beetles belonging to 20 species from 12 genera (Table 1) were collected from August 2003 to October 2006 at 12 sites, elevation between 220 and 1600 m from Vitosha, Maleshevska, Rhodope, Pirin and Osogovo mountains. The nomenclature of bark beetles taxa is given according to Alonso-Zarazaga (2004). Beetles were collected from wind-thrown trees by peeling off the bark and removing the insects from the exposed breeding galleries. Each sample contained beetles from different breeding systems. Between 5 and 241 individuals were collected from each site, for a total of 2.433 beetles.

Collected beetles and pieces of bark were transported to the laboratory. The beetles were refrigerated at 5°C to reduce movement and prevent horizontal transmission of any pathogens. The surviving beetles were dissected as described in Takov *et al.* 2006. Beetles, which died under refrigeration from fungal infections, were checked for conidiospores to determine whether it was a pathogen or a saprophyte. Fresh preparations of the gonads, Malpighian tubules, fat body, and the entire gut were examined for the presence of pathogens under light microscopy (200-1000x). When pathogens were observed, Giemsa stained smears were made of the infected tissues (Weiser 1977). Sizes of cysts, spores and gregarine trophozoites were measured with an ocular micrometer at magnifications of 200 and 400 x.

The $\chi 2$ test was used for statistical calculation of differences in infection prevalences among the different sites (Essi 1987). The biometric characterization of *G. typographi* from *I. typographus* and *I. sexdentatus* was made using Statistica v. 6. The following parameters were calculated: arithmetic mean (Mean); median (M); standard deviation (SD); standard error of the mean (SE); coefficient of variation in % (CV); extreme values (Min and Max); number of examined individuals (N).

Results

Seven pathogen species were recovered from 9 of 20 bark beetle species studied: *I. typographus* Entomopoxvirus (*It*EPV) Wegensteiner, Weiser (Entomopoxviridae), *Malamoeba scolyti* Purrini (Protozoa, Rhizopoda), *Gregarina typographi* Fuchs (Protozoa, Eugregarinida), *Menzbieria chalcographi* Weiser (Protozoa, Neogregarinida), *Chytridiopsis typographi* Weiser (Fungi, Microsporidia), *Nosema*–like microsporidium (Fungi, Microsporidia) and *Beauveria bassiana* Balsamo (Fungi, Hyphomycetes) (Table 2, 3, 6 and 7). *Nosema*–like microsporidium, *M. chalcographi* and *M. scolyti* are new reports for the Bulgarian fauna. *It*EPV, *G. typographi, C. typographi* and *Beauveria bassiana* have been reported from *Ips typographus* in a previous study (Takov *et al.* 2006), *B. bassiana* from *I. sexdentatus, M. scolyti* from *Pytiophtorus pytiographus, Chytridiopsis* cf. *typographi* from *Orthotomicus proximus* and *Cryphalus saltuarius,* and *Gregarina* cf. *typographi* from *Polygraphus subopacus* are newly reported pathogens for these species hosts.

Ips typographus

Three pathogen species were recorded in *Ips typographus* (Table 1). *G. typographi* was found in the midgut lumen of the host in all three samples and *It*EPV was detected in cells of midgut epithelium of *I. typographus* in both study sites. *B. bassiana* infections were observed in some dead beetles in the sample from 12.10.2006 from Artista. The prevalences of these pathogens in 2006 are presented in Table 1. The total prevalence among pathogens did not differ significantly ($p>0.05$).

The morphometric characteristic of *G. typographi* is presented in Table 2. It is seen that the

Table 1. Studied beetles and localities.

Bark beetle	Host plant	Date	Locality	Altitude(m)
Ips typographus	*Picea abies*	12.05.2006	Artista	1450
		12.10.2006	Artista	1450
		12.10.2006	Yavorova polyana	1510
Ips sexdentatus	*Pinus nigra*	7.08.2003	Zheleznitsa	1000
		2.09.2003	Zheleznitsa	1000
		18.09.2003	Tsalim	999
	Pinus sylvestris	1.07.2005	Tsaparevo	940
		17.07.2005	Tsaparevo	940
		3.11.2005	Tsaparevo	820
		22.06.2006	Tsaparevo	820
Ips acuminatus	*P. sylvestris*	18.09.2003	Tsalim	999
		20.09.2004	Garmen	600
		21.10.2004	Sandanski	220
		14.07.2005	Tsaparevo	999
		25.08.2005	Borino	1150
		15.10.2005	Borino	1150
		12.05.2006	Tsaparevo	820
		12.10.2006	Tremostnica	1300
Pytiogenes chalcographus	*P. abies*	27.06.2004	Garmen	600
P. bistridentatus	*P. sylvestris*	15.07.2005	Tsaparevo	530
Orthotomicus proximus	*P. sylvestris*	5.07.2005	Garmen	600
		20.08.2005	Sandanski	220
O. erosus	*P. sylvestris*	15.07.2004	Gorni Lom	500
O. laricis	*P. sylvestris*	1.07.2005	Tsaparevo	940
Pityophthorus pytiographus	*P. abies*	22.04.2004	Yuchbunar	800
		4.07.2004	Borino	1150
		21.08.2004	Vetrovala	1510
Polygraphus subopacus	*P. abies*	30.06.2004	B. Branishte Reserve	1660
P. poligraphus	*P. abies*	21.08.2004	Artista	1450
Cryphalus saltuarius	*P. abies*	13.10.2005	B. Branishte Reserve	1660
Hylurgus ligniperda	*P. sylvestris*	24.06.2006	Tsaparevo	820
Trypodendron lineatum	*P. sylvestris*	9.09.2006	Tsaparevo	940
Carphoborus rossicus	*P. abies*	4.07.2004	Borino	1150
Dryocoetes autographus	*P. abies*	13.10.2005	B. Branishte Reserve	1660
Hylurgops palliatus	*P. abies*	4.10.2004	B. Branishte Reserve	1660
Hylastes attennuatus	*P. sylvestris*	12.07.2005	Tsaparevo	940
H. ater	*P. sylvestris*	20.06.2005	Tsaparevo	940
H. cunicularius	*P. abies*	27.06.2004	Garmen	600

coefficients of variation of all characters are more than 10% which shows that they are variable.

The length frequency distributions (Fig. 1) yielded a bell-shaped curve (normal distribution) and indicated that *G. typographi* was a size-polymorphic species with a length varying in large limits (from 32.5 to 193.8 μm). It has one well-expressed main size class (between 80-140 μm.

Mixed infections caused by *G. typographi* and *It*EPV were observed in some *I. typographus* beetles.

I. sexdentatus

Two pathogens were detected in *I. sexdentatus* (Table 3). The prevalences of pathogens recovered from *I. sexdentatus* are presented in Table 5. The prevalence of *G. typographi* was high and varied between 0% in beetles from Tsaparevo, Maleshevska mountain, in 2005 and 59.3% in beetles collected in 2003 from Zheleznitsa, Vitosha mountain. *B. bassiana* was found only in one sample from Tsaparevo and its total prevalence was significantly lower than the prevalence of the eugregarine ($p<0.001$).

The morphometric characteristic of *G. typographi* from *I. sexdentatus* is presented in Table 4. In this case the coefficients of variation of all characteristics are also more than 10%, which confirms the variability of the eugregarine.

The analysis of length frequency distributions (Fig. 2) also confirms the fact that *G. typographi* is a size-polymorphic species. Similarly to the length of the eugregarine from *I. typographus*, the main size class of the length (100-140 μm) is well expressed and indicates normal distribution.

I. acuminatus

Two pathogen species were observed in *I. acuminatus* (Table 5). We observed gamonts of *G.* cf. *typographi* in the midgut lumen and spores of *C.* cf. *typographi* enclosed in sporophorous vesicles in the midgut cells of the host. The size of gamonts of *G.* cf. *typographi* varied from 50.0 to 106.25 μm in length and 12.5 to 37.5 μm of width.

The prevalences of both pathogens are presented in Table 6. Infection rates of *G.* cf. *typographi* varied from 0% in a sample from Garmen, the Rhodope mountains in 2004 to 40% in a sample from Tsaparevo, Maleshevska mountain, in 2005. The

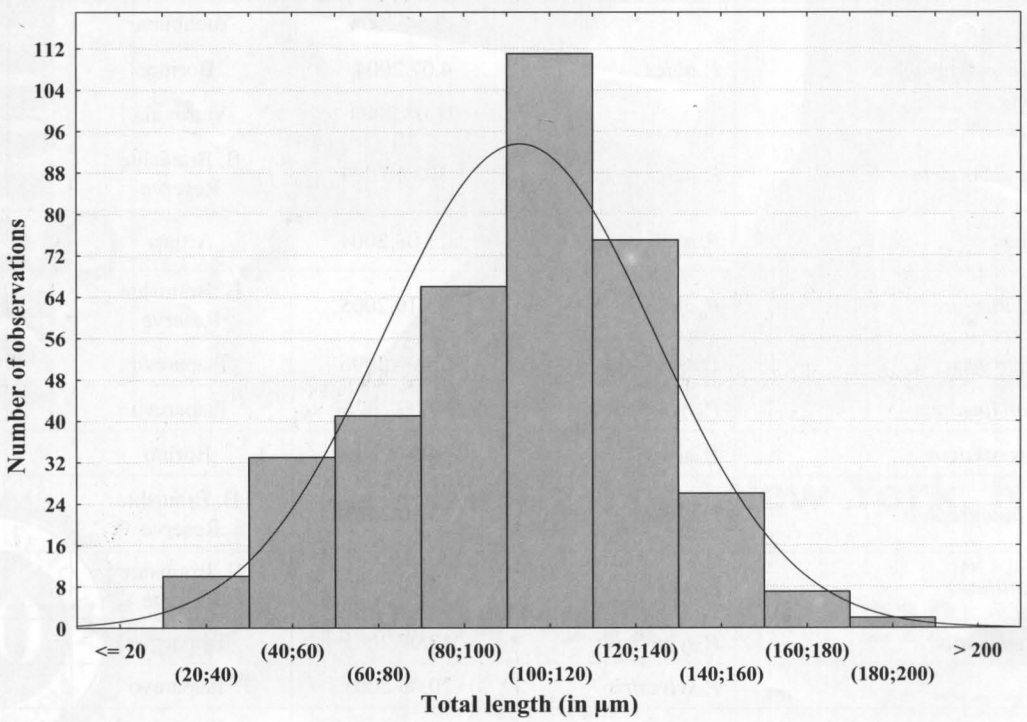

Fig. 1. Length frequency of *Gregarina typographi* from *Ips typographus* (measurements in μm).

Table 2. Pathogens recovered from *Ips typographus* collected in two sites from the Vitosha mountain in Bulgaria.

Site	Date	Number	*G. typographi* N (%)	*B. bassiana* N (%)	*It*EPV N (%)
Artista	12.05.2006	15	2 (13.3)	-	-
Artista	12.10.2006	42	3 (7.1)	3(7.1)	4 (9.5)
Yavorova polyana	12.10.2006	32	4 (12.5)	-	3 (9.4)
Total		89	9 (10.1)	3 (3.4)	7 (7.9)

Abbrevations: N = number of infected individuals; % = percent of infected individuals, *G. typographi* = *Gregarina typographi*, *B. bassiana* = *Beauveria bassiana,* ItEPV= *Ips typographus* entomopoxvirus.

Table 3. Morphometric characteristic of *Gregarina typographi* from *Ips typographus* (measurements in µm).

	Mean	M	SD	SE	CV	Min	Max	N
TL	102.9	107.5	31.7	1.6	30.8	32.5	193.8	371
LP	35.3	37.5	11.0	0.6	31.1	8.8	58.8	371
LD	67.6	67.5	24.3	1.3	35.9	22.5	137.5	371
WP	44.8	41.2	10.7	1.3	23.8	25.0	75.0	371
WD	48.7	46.2	10.6	1.3	21.7	25.0	87.5	371
LP/TL	0.35	0.36	0.07	0.01	20.0	0.14	0.49	371
WP/WD	0.92	0.94	0.1	0.02	10.8	0.63	1.13	371
LP/WP	0.86	0.82	0.2	0.02	23.2	0.42	1.53	371

Abbrevations: arithmetic mean (Mean); median (M); standard deviation (SD); standard error of the mean (SE); coefficient of variation in % (CV); extreme values (Min and Max); number of examined individuals (N), total length (TL), length protomerit (LP), width protomerit (WP), width deutomerit (WD), ratio of LP and TL (LP/TL) WD, ratio of WP and WD (WP/WD), ratio of LP and WP (LP/WP).

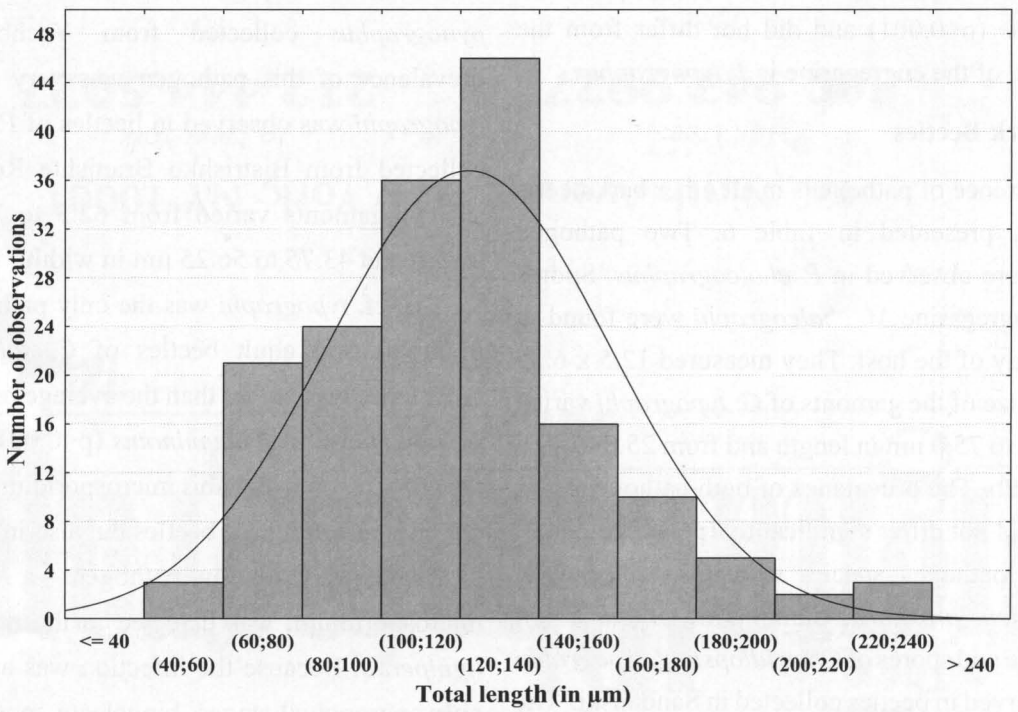

Fig. 2. Histogram showing the length frequency of *G. typographi* from *I. sexdentatus* (measurements in µm).

Table 4. Pathogens recovered from *Ips sexdentatus* collected from the Vitosha, Pirin and Maleshevska mountains.

Site	Collection Date	Number	*G. typographi* N (%)	*B. bassiana* N (%)
Zheleznitsa	7.08.2003	47	15 (31.9)	-
Zheleznitsa	2.09.2003	32	19 (59.3)	-
Tsalim	18.09.2003	157	24 (15.2)	-
Tsaparevo	1.07.2005	5	-	-
Tsaparevo	17.07.2005	20	5 (25.0)	-
Tsaparevo	3.11.2005	37	9 (24.3)	-
Tsaparevo	22.06.06	37	20 (54)	4 (10.8)
Total		335	92 (27.4)	4 (1.2)

Table 5. Morphometric characteristic of *Gregarina typographi* from *Ips sexdentatus* (measurements in μm).

	Mean	M	SD	SE	CV	Min	Max	N
TL	122.05	120.0	36.0	2.77	29.4	55.0	237.5	166
LP	23.3	25.0	7.57	0.6	32.4	10.0	40.0	166
LD	98.8	100.0	31.5	2.44	31.8	40.0	215.	166
WP	39.8	40.0	13.4	1.04	33.6	15.0	90.0	166
WD	73.7	75.0	22.0	1.7	29.8	30.0	110.0	166
LP/TL	0.19	0.19	0.05	0.003	24.7	0.09	0.38	166
WP/WD	0.55	0.52	0.16	0.01	29.0	0.22	1.8	166
LP/WP	0.61	0.6	0.2	0.015	32.7	0.22	1.25	166

Abbrevations: arithmetic mean (Mean); median (M); standard deviation (SD); standard error of the mean (SE); coefficient of variation in % (CV); extreme values (Min and Max); number of examined individuals (N), total length (TL), length protomerit (LP), width protomerit (WP), width deutomerit (WD), ratio of LP and TL (LP/TL) WD, ratio of WP and WD (WP/WD), ratio of LP and WP (LP/WP).

total prevalence of the eugregarine was significantly lower than the prevalence of *G. typographi* in *I. sexdentatus* ($p<0.001$) and did not differ from the prevalence of the eugregarine in *I. typographus.*

Other Bark Beetles

The occurrence of pathogens in all other bark beetle species is presented in Table 6. Two pathogen species were observed in *P. chalcographus*. Spores of the neogregarine *M. chalcographi* were found in the fat body of the host. They measured 12.5 x 6.25 μm. The size of the gamonts of *G. typographi* varied from 62.5 to 75.0 μm in length and from 25.0 to 40.0 μm in width. The prevalence of both pathogens was law and did not differ significantly ($p<0,005$).

Two pathogen species were also detected in *Orthotomicus proximus*. Gamonts of *Gregarina cf. typographi* and spores of *Chytridiopsis cf. typographi* were observed in beetles collected in Sandanski. The prevalence of the pathogens was low.

Cysts of the *Malamoeba scolyti* amoeba were found in the Malpighian tubules of *Pityophthorus pytiographus* collected from Yuchbunar. The prevalence of this pathogen was very low. *G.* cf. *typographi* was observed in beetles of *P. subopacus* collected from Bistrishko Branishte Reserve. The size of gamonts varied from 62.5 to 75.0 μm in length and 43.75 to 56.25 μm in width.

C. cf. *typographi* was the only pathogen found in larvae and adult beetles of *C. saltuarius.* Its prevalence was higher than the average prevalence in *O. proximus* and *I. acuminatus* ($p<0.001$). This was the only case in which this microsporidium was found not only in adult bark beetles but also in larvae.

Infection with new pathogen – a *Nosema*-like microsporidium, was detected in the midgut of *H. ligniperda.* Because the infection was at late stage, only sporogonial stages, binucleate sporoblasts and single binucleate spores of the microsporidium were

Table 6. Pathogens recovered from *Ips acuminatus* collected from the Pirin, Rhodope and Maleshevska mountains.

Site	Date	Number	*Gregarina cf. typographi* N (%)	*Chytridiopsis typographi* N (%)
Tsalim	18.09.2003	30	1 (3.3)	-
Garmen	20.09.2004	38	-	-
Sandanski	21.10.2004	265	35 (13.2)	-
Tsaparevo	14.07.2005	15	6 (40)	1 (6.6)
Borino	25.08.2005	39	4 (10.2)	-
Borino	15.10.2005	48	5 (10.4)	-
Tsaparevo	12.05.2006	5	1 (20.0)	-
Tremosthnitsa	12.10.2006	44	1 (2.3)	5 (11.4)
Total		440	52 (11.8)	6 (1.4)

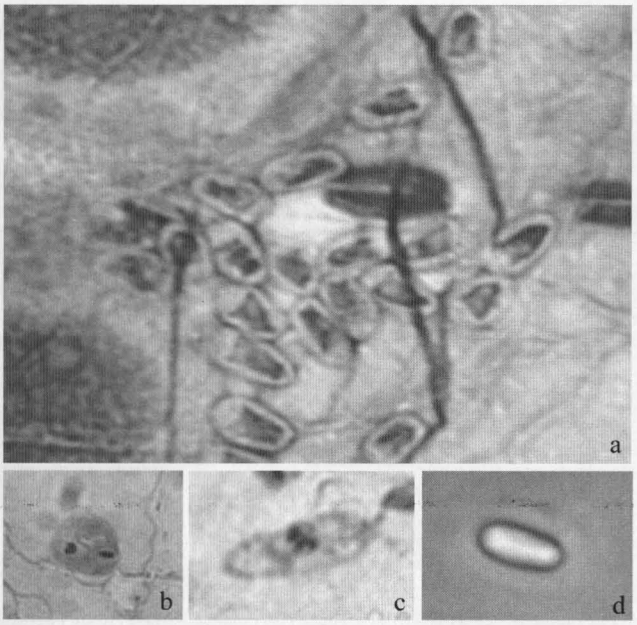

Fig. 3. a. Spores of *Nosema*-like microsporidium from *Hylurgus ligniperda* stained with Giemsa, 2 500 x, b. Sporogonial stage with 2 nuclei, 1900 x., c. Sporoblast with 2 nuclei, 2 500 x., d. Alive spore 2 000 x.

observed (Fig. 3a, 3b, 3c and 3d). The size of fresh spores varied from 4.8 to 7.2 μm in length and 1.8 to 3.30 μm in width. Some midgut cells were filled with spores and were swollen. Four of 20 collected beetles were infected with this microsporidium.

Two pathogens apparently have broad host ranges. *G. typographi* infected six different hosts and *C. typographi* was detected in three hosts.

Discussion

From the seven pathogen species recovered from nine bark beetles collected from 2003-2006, three are first reports for the Bulgarian fauna and five bark beetle species are new hosts for two of the pathogens.

The pathogens recovered from *Ips typographus* are the same species as those reported by Takov *et al.* (2006) for 2003, 2004 and 2005. *Gregarina typographi*, *It* EPV, *Beauveria bassiana* were present again in the beetles collected in 2006. The prevalence of the eugregarine from both sites in the Vitosha mountain was lower than that reported in our previous study (Takov *et al.* 2006) and by Wegensteiner and Weiser (1966).

*It*EPV prevalence in *I. typographus* from both study sites were similar to those reported by Takov *et al.* (2006) for Yavorova Polyana (2003), by Wegensteiner (1996) for beetles collected in Flatz, Gainfarn, Forstheide, Zbiroh in 2004 and by Händel *et al.* (2003) for Tamsweg (1999).

G. typographi was observed in *I. sexdentatus* from all studied sites. Theodorides (1960) first reported this pathogen-host association. The prevalence of the pathogen was similar to that reported by Händel *et al.* (2003) for *I. typographus* in Austrian sites. The total prevalence of *B. bassiana* in *I. sexdentatus* was low, but similar to that in our previous study (Takov *et al.* 2006) for *I. typographus*.

Table 7. Infections of *Pityogenes chalcographus, Pytiophthorus pytiographus, Polygraphus subopacus, C. saltuarius, Orthotomicus proximus* and *Hylurgus ligniperda* from different sites in the Pirin, Osogovo, Rhodope and Vitosha mountains.

Site	Date	Number	*G. cf. typographi* % (N)	*C. cf .typographi* N (%)	*M. chalcographi* N (%)	*M. scolyti* N (%)	*Nosema* sp. N (%)
P. chalcographus							
Garmen	27.06.04	121	3 (2.5)	-	5 (4.1)	-	-
O. proximus							
Garmen	05.07.05	5	-	-	-	-	-
Sandanski	20.08.05	19	1 (5.2)	1 (5.2)	-	-	-
Subtotal		24	1 (4.1)	1 (4.1)	-	-	-
P. pytiographus							
Yuthbunar	22.04.04	241	-	-	-	3 (1.2)	-
Borino	04.07.04	69	-	-	-	-	-
Vetrovala	21.08.04	70	-	-	-	-	-
Subtotal		380	-	-	-	3 (0.78)	-
P. subopacus							
B. Branishte Reserve	30.06.04	176	20 (11.3)	-	-	-	-
C. saltuarius							
B. Branishte Reserve	13.10.05	38	-	4 (10.5)	-	-	-
H. ligniperda							
Tsaparevo	24.06.06	22	-	-	-	-	4 (18.2)

G. cf. *typographi* was the most frequently recovered pathogen in *I. acuminatus*, Zitterer (2002) reported a larger *Gregarina* sp. in *I. acuminatus* collected from Austria and Norway. *Chytridiopsis* sp. (probably *C. typographi*) was reported from this host collected in Austria, the Czech Republic and from Norway by Zitterer (2002).

Händel *et al.* 2003 studied the pathogen complex of *Pityogenes chalcographus* from Austrian spruce stands and reported *G.* cf. *typographi* and *Menzbieria chalcographi* from this host. The prevalence of *G.* cf. *typographi* we recorded was similar to that recorded by Händel *et al.* (2003). The size of the spores of *M. chalcographi* we measured is similar to that reported by Purrini (1980) for this neogregarine found in *P. chalcographus.*

Our finding of *Malamoeba scolyti* in *Pityophthorus. pityographus* is new. This amoeba was originally described by Purrini (1980) in *Dryocetes autographus* and was later recorded from field populations of *Hylurgops palliatus* (Kirchoff, Fuhrer 1985, Händel 2001), *Hylurgops glabatrus* (Haidler 1998), *I. typographus* (Wegensteiner 1994, wegensteiner *et al.* 1996, Händel *et al.* 2003), *Hylastes cunicularis* (2001) and *I. acuminatus* (Zitterer 2002). Our finding confirmed that this pathogen has a broad host range.

The *Nosema*-like microsporidium that we recovered from *Hylurgus ligniperda* is possibly a new species. Several microsporidia belonging to the genus *Nosema* were described from bark beetle species: *N. typographi*, which infects the fat body and the Malpighian tubules of *I. typographus* (Weiser 1955), *N. curvidens* from the fat body, the hypodermis and the connective tissues of *Pityokteines curvidens* (Weiser 1961), *N. scolyti* in the midgut, Malpighian tubules

and haemocytes of *Scolytus scolytus, S. multistriatus, S. pygmeus* and *S. ensifer* and *N. dendroctoni* from the fat body of *Dendroctonus pseudotsugae* (WEISER 1970). Furthermore HÄNDEL *et al.* (2003) observed a *Nosema* sp. in the fat body and midgut epithelium cells of *Hylurgops palliatus*. Our study showed that the *Nosema*-like microsporidium from *H. ligniperda* was located only in the midgut epithelium of its host. For precise identification of this species, however, detailed studies of the pathology, host specificity, ultrastructural characters and rDNA sequence are needed.

Mixed infections, caused by *G. typographi* and *It*EPV, were found in *I. typographus* and in one *I. acuminatus* individual. More species of pathogens were observed in *I. typographus* than in the other bark beetles species we studied, corroborating the findings of HÄNDEL et al. (2003). *G.* cf. *typographi* appears to have the broadest host range of the beetles we examined, followed by *C. typographi,* again corroborated by other previous studies: HAIDLER (1998), HÄNDEL (2002), HÄNDEL *et al.* (2003). *M. chalcographi. M. scolyti* and *Nosema*-like microsporidium appeared to have narrower host ranges; each was found in three host species. None of the pathogens we recovered were restricted to one host species.

The study reported here considers the pathogenic complex of different bark beetles in Bulgaria and suggests that the pathogens we recovered have a relatively broad host range among these sympatric bark beetles. Further investigations to characterize these pathogens using molecular techniques would allow us to better evaluate host-pathogen interactions and better determine pathogen identity and host range. For these cryptic pests the manipulation of naturally occurring parasites may be the most promising method for control.

Acknowledgements: We are especially indebted to Prof. G. Georgiev, Assoc. Prof. S. Draganova, Eng. H. Tomovski, Eng. M. Tatarski, Eng. Jantcho Najdenow, Eng. Tcholakow and E. Tzvetanska for their contributions to this work. We are also grateful to Dr. Leellen Solter for her valuable comments and editing and to the funding provided by the National Science Fund of Bulgaria, grant B 1312/2003.

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Received: 23.11.2006
Accepted: 07.01.2007

Изледвания върху патогени на корояди (Coleoptera:Scolytidae) от иглолистни насаждения в България

Д. Таков, Д. Дойчев, Р. Вагенщейнер, Д. Пиларска

(Резюме)

Изследвани са съставът на комплекса на патогени и тяхното разпространение в различни видове корояди, нападащи иглолистни насаждения. Установени са 7 патогена Ips typographus Entomopoxvirus(It EPV), *Malamoeba scolyti*, *Gregarina typographi*, *Menzbieria chalcographi*, *Chytridiopsis typographi*, *Nosema-like mikrosporidium* и *Baeuveria bassiana*. *Nosema*-like *mikrosporidium*, *M. chalcographi* и *M. scolyti* се съобщават за първи път за българската фауна. *Ips sexdentatus*, *Pityophthorus pityographus*, *Cryphalus saltuarius*, *Orthotomicus proximus* и *Polygraphus subopacus* са нови гостоприемници за *B bassiana*, *M. scolyti*, *Chytridiopsis* cf. *typographi* и *Gregarina* cf. *typographi* съответно. *G. typographi* показва най-широк кръг от гостоприемници следвана от *C. typographi*, *M. chalcographi*, *M. scolyti* и *Nosema*-like *mikrosporidium* имащи по-тесни кръгове на гостоприемници.