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Occurrence of pathogens in populations of *Ips typographus*, *Ips sexdentatus* (Coleoptera, Curculionidae, Scolytinae) and *Hylobius* spp. (Coleoptera, Curculionidae, Curculioninae) from Austria, Poland and France

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Abstract. Occurrence of pathogens in four important forest pest insect species, *Ips typographus, Ips sexdentatus, Hylobius abietis* and *Hylobius pinastri*, from Austria, Poland and France was investigated in 2006–2008. Insects were collected from 46 sampling sites. In total 5,634 living adult beetles were dissected. Some dead adults and some very few dead larvae were inspected for fungal infection. Eight pathogen species (*Ips typographus* entomopoxvirus, *Gregarina typographi*, *Mattesia* sp., *Chytridiopsis typographi*, *Unikaryon montanum*, *Beauveria bassiana*, *Beauveria caledonica* and *Isaria farinosa*) were found in *I. typographus* from the three countries. Four pathogen species were found in *I. sexdentatus* from Austria and France (*Gregarina* cf. *typographi*, *Metschnikowia* cf. *typographi*, *B. bassiana* and *B. caledonica*). Five pathogen species were found in *H. abietis* from Austria and Poland (*Gregarina hylobii*, *Ophryocystis hylobii*, *Nosema hylobii*, *B. bassiana* and *B. brongniartii*) and two in *H. pinastri* from Poland (*G. hylobii* and *O. hylobii*). Some of the pathogen species were found for the first time in one of the three countries or are reported for the first time in one of the investigated beetle species. Differences in occurrence and geographical distribution of these pathogens are discussed with regard to their respective apparent host spectrum and possible ecological requirements.

Key words: bark beetles, pine weevils, diseases, Europe.

INTRODUCTION

The four investigated coleopteran species are pests in conifer forests of Europe: *Ips typographus* (L.), *Ips sexdentatus* (Boerner), *Hylobius abietis* (L.) and *Hylobius pinastri* (Gyllenhal). The most important forest pest insect is the European spruce engraver, *Ips typographus*, which is known to occur in Europe and Asia (Pfeffer *et al.* 1995). This species is able to cause significant mortality to Norway spruce, *Picea abies* (L.), in large areas of central, eastern and northern Europe (Ciesla 2011). *I. sexdentatus*, the six-toothed engraver beetle, occurs in Europe and Asia on *Pinus* sp. (Pfeffer

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et al. 1995). According to Grégoire and Evans (2004) the economic importance of *I. sexdentatus* is considered very variable in Europe, from negligible to highly important, as prominent reported recently from Spain and France (in Etxebeste and Pajares 2011, López and Goldarazena 2012). *Hylobius abietis*, the large pine weevil, is known as an economic pest in various parts of Europe, because it feeds on phloem of young conifers in reforestation areas, leading in some cases to the complete destruction of new plantations (Leather *et al.* 1999, Lempérière and Julien 2003, Ciesla 2011). The related species, *Hylobius pinastri*, is suspected to cause less damage (Eidmann 1974).

The natural enemies of these four beetle species, especially *Ips typographus*, were the subject of investigations from the beginning of the 20th century, and were given an increasing interest in the 1950s. Overview data relating to the situation in Europe appeared rather scarce and referred to mostly *I. typographus* (Wegensteiner 2004, Burjanadze and Goginashvili 2009, Wegensteiner and Weiser 2009, Kereselidze *et al.* 2010, Michalková *et al.* 2012, Wegensteiner *et al.* 2015), presenting evidence of an extensive pathogen species spectrum. As regards *I. sexdentatus*, only very few pathogens are mentioned in the literature (Théodoridès 1960, Wegensteiner *et al.* 2007a, Unal *et al.* 2009, Gokturk *et al.* 2010, Wegensteiner *et al.* 2015). The situation is similar for *Hylobius* spp.: the first identified pathogen in *H. abietis*, *Gregarina hylobii* Fuchs (Sporozoa, Eugregarinida), was described in Germany (Fuchs 1915) and rediscovered more than 65 years later in the same country (Purrini and Ormières 1981). Until now there is a great knowledge gap in geographical distribution of pathogens of the investigated species and about key factors for occurrence of individual pathogen species (Wegensteiner *et al.* 2015).

With regard to this situation, we decided in this work to compare the occurrence and host spectrum of the pathogens in swarming individuals (except entomopathogenic fungi, which were observed on dead insects) of these four coniferous pest species in selected locations within three European countries, Austria, France and Poland and in different altitudes. The sampled locations stand also for some bio-geographic regions as distinguished by the European Environment Agency (2002) plus some transitional regions: Northern continental – Baltic (Poland), transitional Atlantic – continental (Austria), transitional Alpine – Illyric (Austria), western Alps (France) and Atlantic (France) connected also with different climatic situations. Most of the study sites were located in managed forests, only very few were prospected in protected or conservation areas.

MATERIALS AND METHODS

Beetle collection

Areas with *P. abies* and *Pinus* spp. were prospected in several regions in Austria (Lower Austria, Upper Austria, Carinthia, Burgenland), Poland (Podlaskie, Mazowieckie, Wielkopolskie and Lubelskie) and France (Rhône-Alpes, Franche-Comté, Limousin, Centre and Auvergne) (Figure 1). Except Poland, most of the areas prospected in France and in Austria are situated in hilly or mountainous regions. In each area several locations were investigated for the presence of bark beetles and weevils. Locations were selected by chance (accidental findings of infested trees or logs) or following advice of local foresters or forest administration personnel. In any case the majority of trees in the stands circumjacent to the sampling sites were more than 60 years old. The areal size of the forests differed greatly, but was explicit more than one hectare at all sites. Concerning the management status of prospected areas, two sampling sites were within old conservation forests, i.e. site no. 1: Rothwald (IUCN-category Ia) in Austria and site no. 31: Białowieża 8 (UNESCO Nature World Heritage and Biosphere Reserve) in Poland. One more sampling site was located within a recently established conservation forest (since 2002), site no. 3: Hundsau (IUCN-category Ib) in Austria that is next to the Rothwald area. All other sites were within managed forests, even all other sampling sites in Białowieża area were within a distance of two to four kilometres maximum to the strict reserve area, but all these sites are within Natura 2000 area since 2007 (Białowieża sites no. 1 to 7 and 9).

I. typographus was collected in Poland and Austria in 2006, 2007 and 2008, and in France in 2007 and 2008. Beetles were removed directly out of the phloem in many cases; in some situations it was possible to get infested log sections to incubate them in the laboratory (collecting only emerging parental beetles). In one location only (Lambach, Austria) *I. typographus* were collected from pheromone traps. *I. sexdentatus* was collected from logs in Austria in 2006, 2007 and 2008 (in 2008 also from pheromone baited traps), and in France in 2007 and 2008 by cutting them out of their galleries in the phloem. *H. abietis* was collected from spruce bark traps and from excavated roots of *P. abies* stumps (in Austria), from pine trap log-sections or pine trap trunk-discs (in Poland) in 2006, 2007 and 2008, while *H. pinastri* was collected only in Poland in 2006, also from pine trap log-sections or pine trap trunk-discs.

All Austrian, Polish and French locations (latitude and longitude were determined with the World Geodetic System 1984 (WGS 84) in Google Earth satellite view) where specimens of at least one of these four beetle species were collected are listed in Tables 1, 2 and 3.

Pathogen diagnosis

After transport to the laboratory, beetles were stored in Petri dishes together with some small spruce or pine bark pieces in

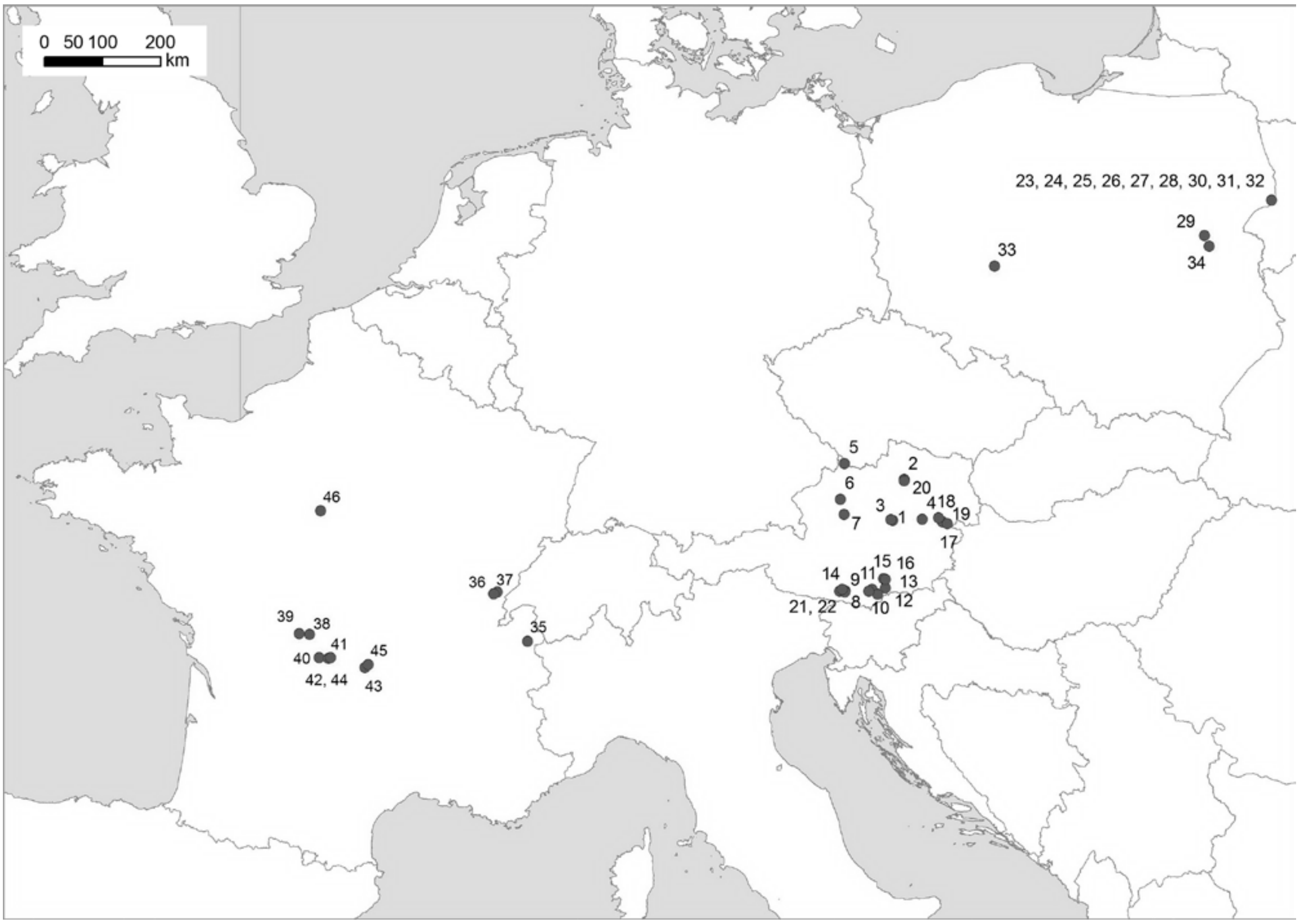

Fig. 1. Sampling sites in Austria (no. 1–22), Poland (no. 23–34) and France (no. 35–46).

an incubator at +15°C (±1.5°C) until they were dissected (maximum seven days). Log sections were incubated in the laboratory at +25°C (±2°C) and under long day conditions (L:D = 18:6) to allow beetle emergence; emerging insects were removed daily for further observations. In some very few occasions, mortality occurred among beetles they were not dissected. Occurrence of pathogens in individually dissected living beetles was recorded in a normal light microscope by the presence of resistant stages like spheroids (entomopoxvirus), spores (neogregarina, microsporidia), trophozoites or gamonts (eugregarina) or asci (ascomycete yeasts). The distribution of all pathogens in individual organs or free in the haemolymph was recorded with exception of bacteria and fungi. After checking fresh smears under the light microscope (at magnification 400 ×) they were dried at room temperature (+22°C ± 1.5°C), fixed with methanol and stained with Giemsa's dye for further observation (measurements with an eyepiece micrometre) under the light microscope (at magnification 1000 ×) (Wegensteiner *et al.* 1996).

Furthermore, special attention was paid to dead beetles bearing symptoms of a fungal infection *in situ*. In all cases these cadavers were stored separately in Petri dishes, and brought to the laboratory, where macroscopic and microscopic observations as well as attempts to isolate the etiological agent were carried out, using standard methods (e.g. Balazy 1966, Goettel and Inglis 1997). Fungal species were then identified on basis of phenotypic and molecular characterization.

Data are expressed in percentages of infection according to the host and location. They can give a first indication about occurrence of various pathogens in the studied beetle species in different European regions, and about possible involved influencing factors. No statistical analysis was carried out, due to unequal number of sampling sites within the three countries, which are not representative for the respective whole country, the varying methods of sampling and different time periods of sampling. In addition, number of collected beetles differed a lot according to sampling sites, and in some cases the number of dissected beetles was quite low.

Table 1. Sampling sites in Austria (No. = number of location; altitude, geographical coordinates = latitude and longitude, federal state) and year of collection (2006, 2007 and 2008) where *Ips typographus* (I.t.), *I. sexdentatus* (I.s.) and *Hylobius abietis* (H.a.) were found respectively.

Locality	No.	Altitude	Latitude (North)	Longitude (East)	Federal state	Year	Species
Rothwald	1.	1100 m	47°46′11″N	15°04′26″E	Lower Austria	2006	I.t.
Hubhof	2.	600 m	48°23′30″N	15°21′10″E	Lower Austria	2007	I.t.
Hundsau	3.	950 m	47°46′38″N	15°02′32″E	Lower Austria	2008	I.t.
Nasswald	4.	720 m	47°44′14″N	15°39′52″E	Lower Austria	2008	I.t.
Aigen-Schlägl	5.	790 m	48°39′55″N	13°58′23″E	Upper Austria	2006	I.t.
Lambach	6.	400 m	48°06′04″N	13°52′18″E	Upper Austria	2006	I.t.
Grünau	7.	590 m	47°46′03″N	13°56′58″E	Upper Austria	2007	I.t.
Treffen	8.	745 m	46°40′25″N	13°51′44″E	Carinthia	2008	I.t.
Ossiach	9.	770 m	46°40′36″N	14°00′11″E	Carinthia	2008	I.t.
St. Georgen im Lavanttal	10.	950 m	46°43′25″N	14°57′15″E	Carinthia	2008	I.t.
Moos	11.	484 m	46°35′52″N	14°46′21″E	Carinthia	2008	I.t.
Grenzgraben	12.	470 m	46°36′13″N	14°43′32″E	Carinthia	2008	I.t.
Unterbergen	13.	552 m	46°33′13″N	14°44′14″E	Carinthia	2008	I.t.
Ossiacher Tauern	14.	860 m	46°40′21″N	14°00′10″E	Carinthia	2008	I.t.
Frantschach	15.	490 m	46°51′58″N	14°52′20″E	Carinthia	2008	I.t.
Prössinggraben	16.	680 m	46°51′02″N	14°55′11″E	Carinthia	2008	I.t.
Lanzenkirchen	17.	299 m	47°45′23″N	16°13′58″E	Lower Austria	2006/07/08	I.s.
Weikersdorf	18.	295 m	47°48′14″N	16°09′44″E	Lower Austria	2008	I.s.
Forchtenstein	19.	400 m	47°42′33″N	16°21′02″E	Burgenland	2007	I.s.
Mühldorf	20.	380 m	48°22′19″N	15°20′46″E	Lower Austria	2008	H.a.
Gerlitzen 1	21.	1560 m	46°41′58″N	13°56′01″E	Carinthia	2007	H.a.
Gerlitzen 2	22.	1576 m	46°41′32″N	13°55′49″E	Carinthia	2007	H.a.

Table 2. Sampling sites in Poland (No. = number of location; altitude, geographical coordinates = latitude and longitude, federal state) and year of collection (2006, 2007 and 2008) where *Ips typographus* (I.t.), *Hylobius abietis* (H.a.) and *H. pinastri* (H.p.) were found respectively.

Locality	No.	Altitude	Latitude (North)	Longitude (East)	Federal state	Year	Beetle species
Białowieża 1	23.	170 m	52°42′04″N	23°53′10″E	Podlaskie	2006	I.t.
Białowieża 2	24.	170 m	52°44′19″N	23°47′11″E	Podlaskie	2006	I.t.
Białowieża 3	25.	170 m	52°42′13″N	23°53′56″E	Podlaskie	2006	I.t.
Białowieża 4	26.	170 m	52°39′58″N	23°45′33″E	Podlaskie	2006	I.t.
Białowieża 5	27.	170 m	52°40′12″N	23°46′12″E	Podlaskie	2006	I.t.
Białowieża 6	28.	170 m	52°43′36″N	23°50′32″E	Podlaskie	2006	I.t.
Siedlce	29.	155 m	52°11′15″N	22°09′48″E	Mazowieckie	2007	I.t.
Białowieża 7	30.	170 m	52°40′59″N	23°47′07″E	Podlaskie	2007	I.t.
Białowieża 8	31.	17 0m	52°43′49″N	23°49′54″E	Podlaskie	2007	I.t.
Białowieża 9	32.	170 m	52°41′30″N	23°49′10″E	Podlaskie	2006/07	H.a./H.p.
Krotoszyn	33.	130 m	51°40′48″N	17°26′03″E	Wielkopolskie	2007	H.a.
Krynka	34.	170 m	52°00′12″N	22°23′25″E	Lubelskie	2008	H.a.

Table 3. Sampling sites in France (No. = number of location; altitude, geographical coordinates = latitude and longitude, region) and year of collection (2007 and 2008) where *Ips typographus* (I.t.) and *I. sexdentatus* (I.s.) were found respectively.

Locality	No.	Altitude	Latitude (North)	Longitude (East)	Region	Year	Beetle species
Combloux	35.	980 m	45°88′11″N	06°62′75″E	Rhône-Alpes	2007	I.t.
Chaux-des-Crotenay	36.	800 m	46°65′53″N	05°94′09″E	Franche-Comté	2007	I.t.
Forêt de la Haute-Joux	37.	860 m	46°69′26″N	06°05′80″E	Franche-Comté	2007	I.t.
Châtelus-le-Marcheix	38.	405 m	46°00′13″N	01°61′61″E	Limousin	2008	I.t.
Les Tenelles	39.	450 m	46°01′28″N	01°36′79″E	Limousin	2008	I.t.
Lacelle	40.	300 m	45°63′45″N	01°83′60″E	Limousin	2008	I.t.
Saint-Merd-les-Oussines	41.	850 m	45°63′09″N	02°03′67″E	Limousin	2008	I.t.
Millevaches 1	42.	800 m	45°64′45″N	02°08′38″E	Limousin	2008	I.t.
Puy de Montchal	43.	1260 m	45°48′80″N	02°88′35″E	Auvergne	2008	I.t.
Millevaches 2	44.	750 m	45°64′34″N	02°09′39″E	Limousin	2008	I.s.
Montredon	45.	1050 m	45°53′29″N	02°96′15″E	Auvergne	2008	I.s.
Olivet/Orléans	46.	125 m	47°49′43″N	01°54′50″E	Centre	2008	I.s.

RESULTS

A total of 4,108 living adult *I. typographus* were dissected during the three years of examinations, 2,614 from Austria (16 sampling sites), 789 from Poland (9 sampling sites) and 705 from France (7 sampling sites). The number of dissected beetles per site varied within a wide range (12 to 470; Table 4). Moreover 84 adult *I. typographus* were found killed by entomopathogenic fungi sometimes in places where no living beetles were noticed (Table 5).

A total of 1,078 living adult *I. sexdentatus* were dissected during the three years, 743 from Austria (3 sampling sites) and 335 from France (3 sampling sites). There were no *I. sexdentatus* observed in Poland. The number of analysed beetles per site ranged from 25 to 285 (Table 6). Furthermore several dead adult *I. sexdentatus* were found infected by entomopathogenic fungi, both in Austria and France.

A total of 397 living adult *H. abietis* and 51 adult *H. pinastri* were analysed during the three years period, 130 *H. abietis* from Austria (3 locations), 267 *H. abietis* from Poland (3 locations), and 51 *H. pinastri* from Poland (1 location). No *Hylobius* spp. were observed in French locations. The number of dissected *H. abietis* per site ranged from 3 to 131 (Table 7). Furthermore, we found 14 adult *H. abietis* and *H. pinastri* dead from fungal infections, and a few dead *H. abietis* larvae *in situ*.

At least one pathogen species was found in beetles collected from every location, except from one site in France for *I. typographus* (Forêt de la Haute-Joux) and two sites for *H. abietis*, one in Poland (Białowieża 9) and one in Austria (Mühldorf).

Pathogens found in *Ips typographus*

During the three-year investigation five different pathogen species except fungi (three species) were found in *I. typographus*: the *I. typographus* entomopoxvirus (*It*EPV); the eugregarine, *Gregarina typographi* Fuchs; the neogregarine, *Mattesia* sp.; the microsporidia *Chytridiopsis typographi* Weiser and *Unikaryon montanum* Weiser, Wegensteiner & Zizka. In addition, symptoms of fungal infections were observed on cadavers in the field. Differences occurred regarding the presence of these various pathogens and their prevalence according to the locations (Tables 4 and 5).

The *It*EPV and *U. montanum* were found in *I. typographus* for the first time in one French site only, in the Alps, while the latter appeared rather common in sampled Polish localities and also in some few Austrian locations. The *It*EPV was found in beetles from four Austrian sites and two French sites, but never in beetles from Poland. *G. typographi* was detected in beetles from all Austrian sites and from seven of the

Table 4. Pathogens (EPV = *It*EPV, G.t. = *G. typographi*, Matt. = *Mattesia* sp., C.t. = *C. typographi*, U.m. = *U. montanum*) found in *Ips typographus* (in %) from different locations (No. of location) in Austria (A), Poland (PL) and France (F) in 2006, 2007 and 2008. N = number of inspected beetles.

Location	No.	Country	Year	N	EPV	G.t.	Matt.	C.t.	U.m.
Rothwald	1.	A	2006	470	0.4	18.9	–	0.2	–
Aigen-Schlägl*	5.	A	2006	328	12.2	0.3	0.6	2.1	–
Lambach**	6.	A	2006	135	–	0.7	–	2.2	0.7
Hubhof*	2.	A	2007	90	–	1.1	–	11.1	–
Grünau	7.	A	2007	276	–	11.6	–	5.1	0.4
Treffen*	8.	A	2008	224	0.4	10.7	–	25.4	0.4
Ossiach*	9.	A	2008	274	–	9.9	–	20.8	–
St. Georgen/ Lavanttal*	10.	A	2008	304	21.4	1.6	–	36.5	–
Hundsau (summer)	3.	A	2008	205	–	57.6	–	3.4	–
Hundsau (fall)	3.	A	2008	290	–	66.9	–	1.0	0.3
Nasswald*	4.	A	2008	18	–	22.2	–	–	–
Białowieża 1	23.	PL	2006	60	–	3.3	–	65.0	1.7
Białowieża 2	24.	PL	2006	37	–	27.0	–	81.1	5.4
Białowieża 3	25.	PL	2006	123	–	8.9	–	7.3	6.5
Białowieża 4	26.	PL	2006	12	–	–	–	83.3	8.3
Białowieża 5	27.	PL	2006	48	–	45.8	–	60.4	6.3
Białowieża 6	28.	PL	2006	110	–	–	–	10.9	10.9
Białowieża 7	30.	PL	2007	200	–	2.0	–	73.5	9.0
Białowieża 8	31.	PL	2007	87	–	4.6	–	81.6	6.9
Siedlce	29.	PL	2007	112	–	0.9	–	42.9	4.5
Combloux	35.	F	2007	135	13.3	15.6	–	15.6	15.6
Chaux-des-Crotenay	36.	F	2007	18	–	5.6	–	–	–
Forêt de la Haute-Joux	37.	F	2007	40	–	–	–	–	–
Les Tenelles	39.	F	2008	45	–	–	–	4.4	–
Lacelle	40.	F	2008	108	–	–	–	0.9	–
Millevaches 1	42.	F	2008	237	–	7.2	–	2.1	–
Puy de Montchal	43.	F	2008	122	4.9	28.7	–	2.5	–

* Emergence of beetles from log section in the lab.
** Beetles from pheromone trap.

nine Polish sampling areas, and it occurred in beetles from four of the seven French sites. The neogregarine *Mattesia* sp. was found only in two beetles from one Austrian location in 2006. *C. typographi* occurred in beetles from nine of the ten Austrian sites, in beetles from all nine locations in Poland, and in beetles from five of the seven French localities. The other microsporidium, *U. montanum*, was detected in beetles from four Austrian sites, in beetles from all nine Polish sampling sites, but in beetles from only one French locality (Table 4).

With regard to infections, *C. typographi* caused highest infection rates (>80% in a few locations inside the Polish forest of Białowieża), and somewhat lower with *G. typographi* (66.9% in one Austrian location). All other pathogens occurred at lower rates, the *It*EPV (0.4–21.4%) and *U. montanum* (0.3–15.6%) infections varied at comparably low values, *Mattesia* sp. only once at 0.6%. Interestingly, *U. montanum* was found in autumn-collected beetles but not in summer-collected ones in one site (Hundsau), and *G. typographi* infection

Table 5. Entomopathogenic fungi found on *Ips typographus* in France (F), Poland (PL) and Austria (A) in 2007 and 2008 (No. = number of location, N = number of dead infected beetles, n = number of isolates).

Location	No.	Country	Year	N	n	Fungal species
Moos	11.	A	2008	8	2	Beauveria bassiana
Grenzgraben	12.	A	2008	5	2	Beauveria bassiana
Unterbergen	13.	A	2008	8	3	Beauveria bassiana
					1	Isaria farinosa
Ossiacher Tauern	14.	A	2008	5	2	Beauveria bassiana
					2	Isaria farinosa
Frantschach	15.	A	2008	5	5	Beauveria bassiana
Prössinggraben	16.	A	2008	10	1	Beauveria bassiana
Białowieża 7	30.	PL	2007	12	5	Beauveria bassiana
Chaux-des-Crotenay	36.	F	2007	2	1	Beauveria bassiana
Forêt de la Haute-Joux	37.	F	2007	3	1	Beauveria bassiana
Châtelus-le-Marcheix	38.	F	2008	6	0	Beauveria bassiana
Les Tenelles	39.	F	2008	7	2	Beauveria bassiana
					2	Beauveria caledonica
Lacelle	40.	F	2008	5	1	Beauveria bassiana
Saint-Merd-les-Oussines	41.	F	2008	4	1	Beauveria caledonica
					1	Isaria farinosa
Puy de Montchal	43.	F	2008	4	2	Beauveria bassiana

Table 6. Entomopathogens (EPV = *It*EPV, G.t. = *G. typographi*, Matt. = *Mattesia* sp., C.t. = *C. typographi*, U.m. = *U. montanum*, B.b. = *Beauveria bassiana,* B.c. = *Beauveria caledonica*, I.s. = *Isaria farinosa*) found in *Ips typographus* from Austria, Poland and France; first evidence of a pathogen in a country is marked with an asterisk.

Country	EPV	G.t.	Matt.	C.t.	U.m.	B.b.	B.c.	I.f.
Austria	+	+	+	+	+	+	–	+*
Poland	–	+	–	+	+	+	–	–
France	+*	+	–	+	+*	+	+*	+*

Table 7. Pathogens (G.t. = *Gregarina* cf. *typographi* and M.t. = *Metschnikowia* cf. *typographi*) in *Ips sexdentatus* (in %) from different locations (No. of location) in Austria (A) and France (F) in the years 2007 and 2008. N = number of inspected beetles.

Location	No.	Country	Year	N	G.t.	M.t.
Lanzenkirchen	17.	A	2006	150	36.0	18.0
Lanzenkirchen			2007	212	32.1	20.8
Lanzenkirchen			2008	25	36.0	–
Weikersdorf*	18.	A	2008	285	41.8	8.1
Forchtenstein	19.	A	2007	71	26.8	8.5
Millevaches 2	44.	F	2008	128	66.4	–
Montredon	45.	F	2008	110	39.1	–
Olivet/Orléans	46.	F	2008	97	76.3	1.0

* Beetles from pheromone traps.

rates were significantly higher in autumn-collected beetles compared to summer collected beetles from the same site. Pathogen species spectrum was widest, with four different pathogen species, in the beetles from two Austrian sites (Aigen-Schlägl 2006 and Treffen 2008) and from one French site (Combloux, 2007) (Table 4).

Whereas no *It*EPV infections were observed in Poland, lower *It*EPV infection rates occurred in beetles from sites 0–500 m altitude from Austria and France, compared to higher altitude-sites (except Austria 1001–1500 m). Noticeable lowest *G. typographi* infection rates were found in sites 0–500 m altitude from all three countries compared to higher altitude, but also in France 501–1000 m infection rates were lower compared to higher located sites. Controversial infection rates were recorded with *C. typographi*, highest in Poland 0–500 m and lowest in Austria and France 0–500 m. Further, controversial infection rates were found with *U. montanum* too, highest in Poland 0–500 m (and France 501–1000 m) compared to Austria and France 0–500 m (and other altitudes).

With regard to infections in different bio-geographic regions it was conspicuous that no *It*EPV infection was found in Poland (northern continental), and low *It*EPV infection rates in sites from north of the eastern Alps (transitional Atlantic – continental) and from western Alps. By contrast, *It*EPV infection rates in sampling sites from south of the eastern Alps (transitional Alpine – Illyric) were considerably higher. In case of *G. typographi* only beetles from the sites north of the eastern Alps (transitional Atlantic – continental) showed higher infection rates compared to all other bio-geographic regions. On the other hand highest infection rates were recorded for *C. typographi* in northern continental – Baltic conditions (Poland), but also in sites from south of the eastern Alps (transitional Alpine – Illyric), and both differed from sites in the other bio-geographic regions. The highest *U. montanum* infection rate was found in beetles from one site in the western Alps (Combloux) and in one northern continental – Baltic site (Bialowieza 6) (Table 4). However, when including all sites from a bio-geographic region the highest *U. montanum* infection rates were observed in beetles from northern continental – Baltic sites (Poland), and lowest was this infection in sites from north (transitional Atlantic – continental) and south of the eastern Alps (transitional Alpine – Illyric) in Austria (Figure 2).

The differences in pathogen infection rates in beetles from protected forests and from managed forests were very variable (Table 4).

Besides several pathogens in living beetles, individuals killed by an entomopathogenic fungus were found in Austrian, Polish and French locations (Table 5). Infections with the Ascomycete *Beauveria bassiana* (Balsamo) Vuillemin occurred in several regions in France but only in some of the southern regions in Austria (locations 11–16) and in one Polish location (Białowieża 7). Two further Ascomycete species were observed but even more rarely: *Beauveria caledonica* Bissett & Widden in two French locations, and *Isaria farinosa* (Holmskjold) Fries, in two Austrian locations and in one French location only (Table 5).

Some of the fungi, microsporidia and protozoa were found infecting *I. typographus* for the first time in some of the three countries (Table 6).

Pathogens found in *Ips sexdentatus*

During the three-year investigations the eugregarine, *Gregarina* cf. *typographi* Théodoridès and the ascomycetous yeast *Metschnikowia* cf. *typographi* Wegensteiner, Pernek & Weiser, were found in dissected *I. sexdentatus* in Austria and France, and fungal growth (mycelium or conidia) was observed on cadavers also. Confer (cf.) is used for these pathogen species as it is not clear if it is the same or a different pathogen species. Even measurements of gamonts and asci were within the same range as described in the type host, *I. typographus*.

G. cf. *typographi* was found in *I. sexdentatus* from all locations in Austria in 2006, 2007 and 2008, and in France (2008). Infection rates with the gregarine were always relatively high, with highest level in Millevaches 2 and Olivet/Orléans. *M.* cf. *typographi* was found in beetles from all three Austrian sites (Lanzenkirchen, Forchtenstein, Weikersdorf) and in beetles from one French location (Olivet/Orléans). *M.* cf. *typographi* was not observed in *I. sexdentatus* in France yet. In Lanzenkirchen the yeast generated relatively high infection rates in 2006 and 2007 but was not found in 2008. Infection rates were globally higher in all three Austrian sites (transitional Atlantic – continental, with Pannonian influenced climate) compared to the French location (Atlantic climate). Comparing the number of infected *I. sexdentatus* from Austria and France, more *G.* cf. *typographi* infected beetles were observed in France and more *M.* cf. *typographi* infected beetles in Austria (Table 7).

In addition, two entomopathogenic fungi were found infecting *I. sexdentatus* in one French location and in one Austrian location. In France (Montredon) *B. bas-*

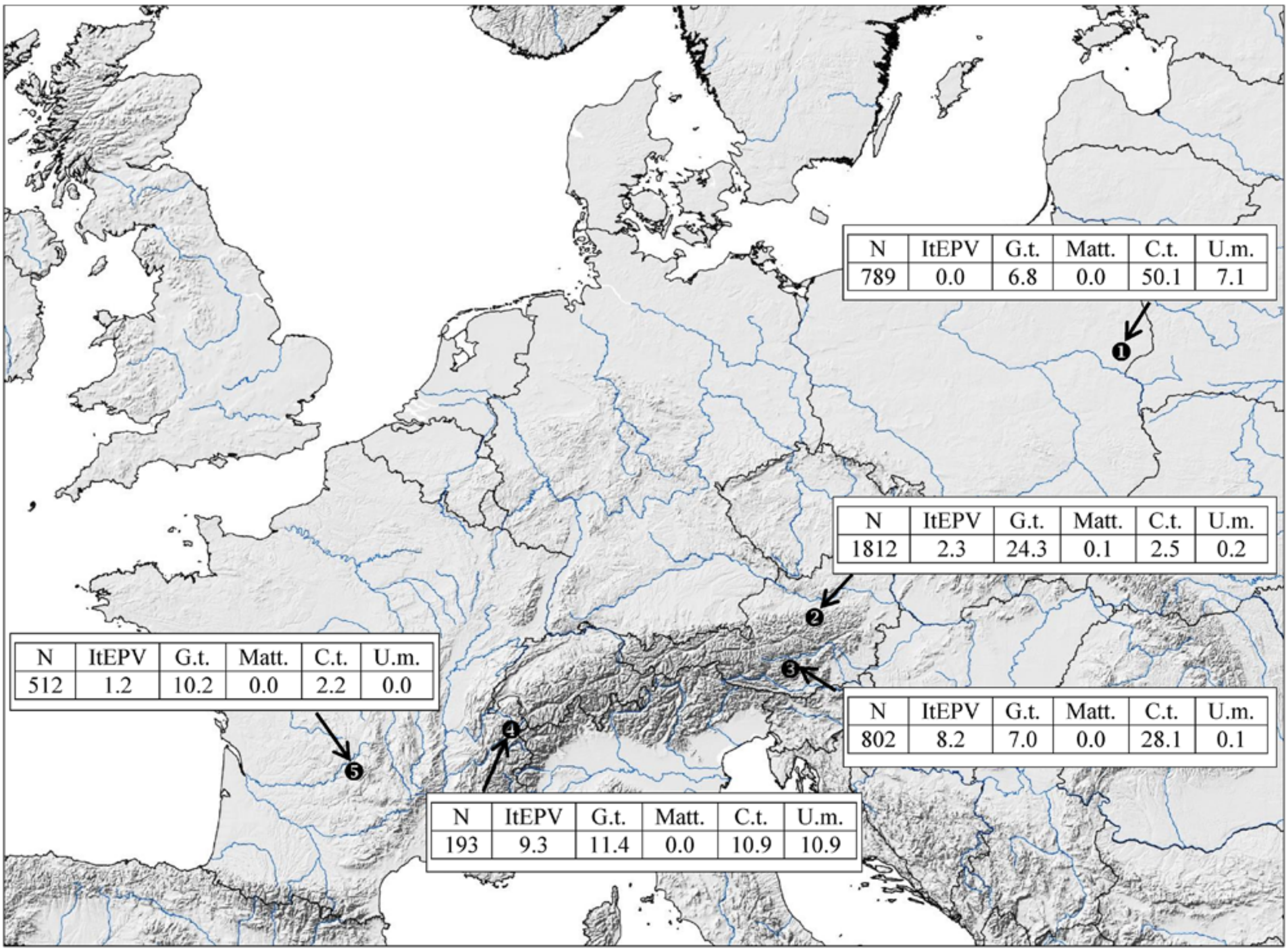

Fig. 2. Pathogens in *I. typographus* from different bio-geographic regions in Europe: ❶ Northern continental – Baltic, ❷ North-eastern Alps (transitional Atlantic – continental), ❸ South-eastern Alps (transitional Alpine – Illyric), ❹ Western Alps and ❺ Atlantic.

siana and *B. caledonica* were observed together in the same beetle population (in 2008), while only *B. bassiana* was found in Austria (Weikersdorf) (in 2008). This is the first reported infection of *I. sexdentatus* by *B. bassiana* in France as well as in Austria, and the first mention of *B. caledonica* infecting *I. sexdentatus* in Europe.

Pathogens found in *Hylobius abietis* and *Hylobius pinastri*

The eugregarine *Gregarina hylobii* Fuchs, the neogregarine *Ophryocystis hylobii* Purrini & Ormières, and the microsporidium *Nosema hylobii* Purrini, were found in *Hylobius abietis* from some few locations in Austria and in Poland, *Gregarina* cf. *hylobii* and *Ophryocystis* cf. *hylobii* in *Hylobius pinastri* from one location in Poland (Table 8). All the pathogens found in *H. abietis* are reported for the first time for Austria and Poland, moreover the pathogens in *H. pinastri* were never reported before.

G. hylobii was observed in both *Hylobius* species in Białowieża 9 and in *H. abietis* from two Austrian locations (Gerlitzen 1 and 2). *O. hylobii* was found in both *Hylobius* species (in Białowieża 9), in *H. abietis* from three more locations in Poland and in *H. abietis* from two Austrian sites. The microsporidium *N. hylobii* was observed in *H. abietis* from two Polish locations only. *G. hylobii* infection rates were relatively low in *H. abietis*; on the other hand *G. hylobii* occurred more frequently in *H. pinastri* from Białowieża (2006) compared to *H. abietis*. *O. hylobii* was found in a high per-

Table 8. Pathogens (G.h. = *G. hylobii*, O.h. = *O. hylobii,* N.h. = *N. hylobii)* in *Hylobius abietis* and in *H. pinastri* (in %) from different locations (No. of location) in Austria (A) and Poland (PL) in the years 2006, 2007 and 2008. N = number of inspected beetles.

Location	No.	Country	Year	N	G.h.	O.h.	N.h.
Mühldorf/*H. abietis*	20.	A	2008	10	–	–	–
Gerlitzen 1/*H. abietis*	21.	A	2007	60	1.7	1.7	–
Gerlitzen 2/*H. abietis*	22.	A	2007	60	6.7	10.0	–
Białowieża 9/*H. abietis*	32.	PL	2006	113	2.7	17.7	0.9
Białowieża 9/*H. abietis*	32.		2007	3	–	–	–
Białowieża 9/*H. pinastri*	32.	PL	2006	51	29.4	7.8	–
Krotoszyn/*H. abietis*	33.	PL	2007	131	–	4.6	3.1
Krynka/*H. abietis*	34.	PL	2008	20	–	30.0	–

centage of *H. abietis* from Krynka in Poland (2008) and also from Białowieża 9 (2006), and less abundant in beetles from all other locations. Highest pathogen diversity was recorded in beetles from one Polish site (Białowieża 9; 2006) with three different pathogen species (Table 8).

In addition to sporozoa and microsporidia the fungus *B. bassiana* was found infecting adult specimens of *H. abietis* in Białowieża 9 and Krotoszyn (Poland), and Gerlitzen 2 (Austria). Besides these findings, completely new were two larvae of *H. abietis* killed *in situ* (in their galleries in the roots) by *Beauveria brongniartii* (Saccardo) Petch in the latter locality.

DISCUSSION

Overall, the data presented here concerning the respective occurrence of *I. typographus*, *I. sexdentatus* and *H. abietis* underline the importance of the former coleopteran species, but very little is known about the occurrence of *H. pinastri*, a species mentioned as very rare in Germany, Sachsen-Anhalt (Schneider 2004) and in Austria (C. Holzschuh pers. comm.).

As already pointed out by Wegensteiner (2004), Wegensteiner *et al.* (2007a, b) and Wegensteiner *et al.* (2015), the widest range of pathogens was observed in the European spruce bark beetle, *I. typographus*: virus (*It*EPV), sporozoa (*Gregarina typographi* and *Mattesia* sp.), microspora (*Chytridiopsis typographi* and *Unikaryon montanum*), and fungi (*Beauveria bassiana*, *B. caledonica* and *Isaria farinosa*) but it must be mentioned that the pathogen complex of *I. typographus* appears to be better studied until now than that of the other beetle species (Wegensteiner 2004, Kenis *et al.* 2004). In general we observed the same species, *G. typographi* and *C. typographi* being most dominant as shown in earlier studies (Wegensteiner *et al.* 1996, Wegensteiner and Weiser 2009). The wide distribution of *G. typographi* might be due to the flight stimulating effect on the (pheromone trap collected) bark beetle hosts (Wegensteiner *et al.* 2010). On the other hand the wide distribution of *C. typographi* is unclear, maybe as a consequence of relatively low virulence.

All the other pathogens known from *I. typographus*, the rhizopodan species *Malamoeba scolyti* Purrini (Wegensteiner 1994) and the neogregarine *Menzbieria chalcographi* Weiser both first described in *I. typographus* from Austria (Wegensteiner and Weiser 2004), the microsporidium *Nosema typographi* Weiser recorded in beetles from Austria, Czech Republic and Germany (Weiser *et al.* 1997), the yeast *Metschnikowia typographi* Weiser, Wegensteiner, Händel & Žižka reported from Austria and Finland (Weiser *et al.* 2003) and the fungus *Metarhizium anisopliae* var. *anisopliae* (Metschnikoff) Sorokin detected in Switzerland (Keller *et al.* 2004) were not found.

Concerning *I. sexdentatus* we found most of the pathogens known from that species. As expected, we observed in a few French localities specimens infected with *G.* cf. *typographi*, as first observed in that country by Théodoridès (1960). When considering the morphologically identical *G. typographi* in *I. typographus* and *G.* cf. *typographi* in *I. sexdentatus*, a possible pathogen transfer is unclear because these two beetle species develop normally on different tree species; however, the precise identity of *G. typographi* in *I. typographus* and of *G.* cf. *typographi* (Geus 1969) or *M. typographi* in *I. typographus* and *M.* cf. *typographi* in *I. sexdentatus* is debatable and an in-depth comparison of the respective characteristics of these pathogens is still needed. Electron microscopy was not used because the method is no sufficient for exact discrimination at least of these species, whereas there is no reproducible molecular method available for these pathogen species at the moment (for some few sporozoan and microsporidian species are only first attempts known).

The well-known entomopathogenic fungus *B. bassiana* was found on *I. typographus*, *I. sexdentatus* and *H. abietis* as well, but appeared more frequently on the former species. This fungus was known as pathogen for *I. typographus* and *I. duplicatus* in Europe since a study by Karpiński (1935) in Poland. The occurrence of *B. bassiana* on *I. typographus* in different areas in France is in accordance with first observations made in Haute-Vienne in 1985 (B. Papierok and G. Lempérière unpublished) but in the other two countries it unexpectedly occurred on that beetle species only in locations in southern Austria (in Carinthia) and in one location in Poland (Białowieża 7). Until now the occurrence of *B. bassiana* (and other fungi) was reported in *I. sexdentatus* from Bulgaria only (Draganova *et al.* 2010, Takov *et al.* 2012). Two other entomopathogenic fungal species appeared less common. *B. caledonica* was observed on *I. typographus* in two French locations only, the second mention of the fungus in populations of *I. typographus* after Kirschner (2001) in Germany. Interestingly the species was found also on *I. sexdentatus* in one French location, together with *B. bassiana* from the same cadaver. The third fungal species, *I. farinosa,* whose presence in European populations of bark beetles was rarely mentioned in the literature until now (Neužilova 1956, Bałazy *et al.* 1967, Kirschner 2001, Landa *et al.* 2001, Draganova *et al.* 2010), was observed on *I. typographus* on a few occasions in France and Austria.

The current knowledge about pathogens in *H. abietis* is very limited (Kenis *et al.* 2004), but our investigations resulted mainly in new data about the pathogen distribution. The three pathogen species we observed in *H. abietis* adults from Austria and Poland, *G. hylobii*, *O. hylobii*, and *N. hylobii*, were known until now from *H. abietis* adults in Germany only (Fuchs 1915, Purrini 1981, Purrini and Ormières 1981). Moreover, it is the first time that *G. hylobii* and *O. hylobii* were found in *H. pinastri*. With regard to fungi infecting *Hylobius* spp., we observed in Poland and in Austria adult *H. abietis* killed by *B. bassiana*, which until now was known on *H. abietis* adults and larvae only from Sweden (Gerdin 1977). Furthermore, nothing was known about the occurrence of *B. brongniartii* on *H. abietis* larvae. However, we did not find on *H. abietis*, either *B. caledonica*, which was found on that beetle in northern UK and Ireland (Glare *et al.* 2008), or a recently described entomophthoralean species, *Tarichium hylobii* Keller, Weiser & Wegensteiner, infecting larvae of *H. abietis* in Czech Republic (Keller *et al.* 2009).

As regards ecological considerations, some differences in pathogen occurrence and prevalence may refer to the bio-geographic region and specific climatic situations of the beetles' origin. Data on the occurrence of pathogens in *I. typographus* brought evidence of a very prevalent distribution of two pathogen species in all the different bio-geographic regions (*G. typographi* and *C. typographi*), which could result from a low virulence of these pathogens. Two other pathogen species were found in several regions, but not in all (*It*EPV and *U. montanum*). The possible influence of the bio-geographic situation is supported by the more frequent occurrence of the *It*EPV in locations south of the eastern Alps (in Austria) and by *C. typographi* and *U. montanum* in beetles from transitional northern continental – Baltic locations (in Poland). Furthermore, there was one pathogen species, *Mattesia* sp., which occurred exclusively in one bio-geographic region (transitional Atlantic – continental).

Various other factors could be involved influencing occurrence and prevalence of pathogens beside differences in the temperature requirements of the various pathogens, density of beetles' tree infestation and number of developing larvae and callow adults, which may cause stress and competition for food resulting in an increased risk for frequent contact of bark beetles and therefore taking an increased risk of getting infected (Wegensteiner and Weiser 1996a, b). The widely distributed pathogen species can be suspected to be active within a wide range of climatic conditions, whereas some species can be more sensitive. Unfortunately nothing is known about the climatic requirements of these pathogens and about their virulence and about effects on their hosts.

The voltinism in a bark beetle population, which is known to be variable depending on geographic latitude and altitude (Wermelinger 2004, Faccoli 2009), may also have an effect on pathogen prevalence related to duration of pathogen development. Anyway, our data showed some trends which confirm previous observations; for instance, higher *G. typographi* infection rates observed in fall-collected compared to spring collected beetles from the location Hundsau are in accordance with previous results (Lukášová and Holuša 2011), which was interpreted as horiziontal transmission in the nuptial chamber of beetles. On the other hand, most of the various pathogen infection rates found in *I. typographus* in our study are higher with increasing altitude except the *C. typographi* rates, which does not correspond with previous findings (Gasperl and Wegensteiner 2012).

Pathogen species diversity in *I. typographus* was even higher in some few managed forests compared to protected forests. The infection rates were also very variable comparing beetles from such two situations. Most probably the permanent presence of host trees suitable for bark beetle breeding is fulfilled in protected forests and in managed forests as well, and therefore the presence of beetle-hosts for the pathogens is guaranteed too. In addition, flight abilities allow beetles migration even over relative long distances (Wermelinger 2004).

In further studies, besides the development of molecular studies to clarify reliably the taxonomic status of some of the observed pathogens, exact registration of climatic conditions in the field will be necessary, in order to identify precisely the influence of climatic conditions on differences in pathogen occurrence. A better knowledge of the ecological requirements of the pathogen species of bark beetles and weevils in the field should allow a better estimation about their potential for biological control of these coniferous pests.

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