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Polygraphus grandiclava (Coleoptera: Curculionidae) collected from pine and cherry trees: A phylogenetic analysis

DIMITRIOS AVTZIS[1], MILOŠ KNÍŽEK[2], KLAUS HELLRIGL[3] and CHRISTIAN STAUFFER[1]

[1] Institute of Forest Entomology, Forest Pathology & Forest Protection, Department of Forest and Wood Sciences, Boku, University of Natural Resources and Applied Life Sciences, Vienna, Austria; e-mail: dimitrios.avtzis@boku.ac.at

[2] Forestry and Game Management Research Institute, Jíloviště-Strnady, Czech Republic

[3] Wolkensteinstraße 83, Brixen, Italy

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Abstract. *Polygraphus grandiclava* (Thomson, 1886) is a unique scolytid species in that it infests both Pinaceae and Rosaceae. The utilization of such different host trees lead to the designation of two species at the beginning of the last century. Later on, these two species were synonymised. Here we investigated the genetic identity of populations collected from pine and cherry trees by sequencing a partial region of the mitochondrial COI gene. The phylogenetic study presented reveals no indication of host-induced differentiation within the mitochondrial sequences of the populations collected from the two host plants.

INTRODUCTION

Allopatric speciation is thought to be the most frequent mode of speciation (Mayr, 1963) as the occurrence of geographic barriers between populations present evident barriers to gene flow (Feder et al., 2005). For a long time sympatric speciation was considered to play a minor role in evolution (e.g. Berlocher & Feder, 2002) but since the first report of host race formation (Bush, 1969) support for this concept has increased (Via, 1999; Dres & Mallet, 2002; Barluenga et al., 2006; Savolainen et al., 2006). In insects, sympatric speciation often involves adaptation to host plants within the same geographic area, something that facilitates the accumulation of incompatible alleles giving rise to isolation (Turelli et al., 2001). Similarities in the secondary metabolic compounds of the two host species play a major role in host shift (Ehrlich & Raven, 1964).

The bark beetle *Polygraphus grandiclava* (Thomson, 1886) is unique among European bark beetles, since in addition to pine, it commonly infests cherry trees, *Prunus avium* (L.) Moench and *Prunus vulgaris* Mill. (Pfeffer, 1995). The infestation of these distantly related tree species belonging to two different families of plants – Pinaceae and Rosaceae – by *P. grandiclava* triggered the interest of forest entomologists at the beginning of the last century. Seitner (1911) studied *P. grandiclava* on *Pinus cembra* L. and based on a morphological analysis described a new species, *Pseudopolygraphus cembrae*, distinct from the species on *P. avium*, *Pseudopolygraphus grandiclava* (Thomson, 1886). Within a few years all these species were synonymised, first to *Lepisomus* Kirby, 1837 (Hopkins, 1915) and later due to strong morphological similarities and the wide intraspecific variation of particular species, they were transferred to the genus *Polygraphus* Erichson, 1836 (Swaine, 1918). *Polygraphus cembrae* (Seitner, 1911) was synonymised to *Polygraphus grandiclava* by Schedl (1934).

The objective of this study was to determine the phylogenetic relationships of *P. grandiclava* populations collected from pine and cherry trees in several geographic regions. A partial region of the mitochondrial COI gene was sequenced and analyzed using a phylogenetic and cladistic approach. In this way it was hoped that the evolutionary background of the host races of *P. grandiclava* would be clarified and a first insight into their phylogeographic pattern revealed.

MATERIAL AND METHODS

P. grandiclava adults were collected from under the bark of *Pinus strobus*, *Pinus cembra*, and *Cerasus avium* at six different localities between 2002 and 2005 (Table 1). Due to the matrilineal inheritance of mtDNA, only one individual per mother gallery was used in the analyses in order to avoid biased haplotype diversity. Specimens for DNA analysis were stored in 96% ethanol at –20°C.

Insect DNA was extracted using the GenElute™ Kit (Sigma, St. Louis, USA) following the protocol of the manufacturer and an amplicon of 663 bp from the 5' end mitochondrial COI gene was polymerized with primers UEA5 and UEA10 (Lunt et al., 1996). Details of the extraction and PCR, PCR purification and sequencing can be found in Avtzis et al. (2008). The sequences were aligned by eye and haplotypes were put in the GenBank (EU428829–EU428842). *Polygraphus polygraphus* (EU428842) infesting *Picea abies* was used as an outgroup species in the phylogenetic reconstruction. A Neighbour-Joining (NJ) approach (Saitu & Nei, 1987) was applied to construct a tree from the pairwise distances that were estimated using the substitution model of Tamura & Nei (1993). The robustness of the NJ tree was assessed by 1000 bootstrap replicates. A maximum parsimony (MP) approach was chosen performing heuristic MP searches using 1000 random-addition sequence replicates and exploring tree space using Tree Bisection and Reconnection (TBR) branch swapping. Bootstrapping was performed using a heuristic search (1000 random-addition-sequence replicates, TBR branch swapping) and 1000 pseudoreplicates.

Further, a Bayesian-based inference was performed with MrBayes version 3.1.1 (Ronquist & Huelsenbeck, 2003) using the nucleotide substitution model defined by the Akaike Information Criterion (Akaike, 1974) as implemented in MrModeltest v2.1 (Nylander, 2004). Hasegawa-Kishino-Yano model (HKY; Hasegawa et al., 1985) with rate heterogeneity (Yang, 1993) (a = 0.0858) was found to be the most appropriate model for two partitions, whereas the General Time Reversible (GTR) was chosen for the third partition (Rodriguez et al., 1990). How-

TABLE 1. Details of the six locations where *Polygraphus grandiclava* were collected and the distribution of the individuals in the three clades (A, B, C). Collection period indicates the time when adult beetles were collected from under the bark of trees in the field.

	Country (abbreviation)	Province – City	Collector	Host tree	Collection period	Clade assignment		
						A	B	C
1	Austria (AT1)	North Tyrol – Hall i. Tirol	M. Kahlen	*P. avium*	06/2003	0	9	0
2	Austria (AT2)	Burgenland – Winden	P. Zabransky	*P. avium*	05/2003	0	10	0
3	Austria (AT3)	Kärnten – Turracher Höhe	D. Avtzis & C. Stauffer	*P. avium*	06/2005	0	0	5
4	Austria (AT4)	Kärnten – Gnesau	D. Avtzis & C. Stauffer	*P. cembra*	06/2005	0	0	6
5	Italy (IT)	South-Tyrol – Bressanone	K. Hellrigl	*P. strobus*	01/2002	15	0	0
6	Czech Rep. (CR)	Bohemia – České Budějovice	M. Knizek	*P. avium*	07/2003	0	2	0

ever, general forms of these models were used in the Bayesian analysis (*nst* = 2, *nst* = 2 and *nst* = 6 for each of the three partitions) since there is a specific recommendation against the use of fixed priors for *a* and *I* in the online manual of MrBayes (http://mrbayes.csit.fsu.edu/wiki/index.php/Tutorial#Specifying_a_Model) as well as in the output file of the MrModeltest v2.1 (Nylander, 2004), for a more efficiently exploration of the different values of these parameters. The number of generations was set to 5,000,000 with a sampling frequency of 100 generations in dual running process. After 1,700,000 generations, stationarity was achieved; the average standard deviation of split frequencies ranged between 0.00343 and 0.0015. The last 3,300 trees of each run were used to compute a majority rule consensus tree and clade posterior probabilities.

Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) as implemented in Arlequin v3.0 (Excoffier et al., 2005) was applied in order to analyze the genetic structure and the causes of divergence within populations of *P. grandiclava*. AMOVA was performed using three different hierarchical grouping options: "by region", "by host" and "by clade". In the option "by region", individuals were nested within host plant and then nested within locality, whereas the option "by host" divided individuals into groups according to their locality and then host preference. Additionally, individuals were also grouped according to clade assignment ("by clade" option).

RESULTS AND DISCUSSION

47 *P. grandiclava* individuals found on *Pinus* and *Prunus*, from six locations, were sequenced. Analysis of the partial COI gene yielded 13 haplotypes (HT) with 34 polymorphic sites, 30 of which were parsimony-informative. Three HT were found only once, whereas HT3 and HT13 were the most frequent with 11 and 8 individuals, respectively (Fig. 1). A maximum sequence divergence of 4.32% was found between HT5 and HT11.

NJ, MP (both not shown) as well as Bayesian statistics yielded phylogenetic trees with similar topologies (Fig. 1), with minor differences in the placement of some HT within the major clades as well as slight differences in the support values. Consequently in all phylogenetic approaches clade A contained individuals infesting *P. strobus*, clade B individuals infesting *P. avium* and clade C individuals infesting both pine and cherry trees. It can thus be concluded from this study that the utilization of different hosts by *P. grandiclava* has not affected its genetic structure.

This outcome is further supported by the results of AMOVA (Table 2), where different grouping options were tested. AMOVA revealed that the grouping option "by region", which divided individuals according to their geographic origin namely Czech Republic, Italy, and Austria, had no effect on the genetic structure. Similarly grouping "by host" had little influence on the genetic variation. Only 12% of the total variation was assigned to the "among groups" option, whereas the other two sources of variation accounted for more than 85% of the variation (Table 2). The results were different when individuals were grouped according to their assignment to clades ("by clades" option). This analysis attributed the variation predominantly to the "among groups" differentiation (90.83%, $P < 0.001$) with a minor influence of the other two sources of variation (Table 2). Thus variation among European populations of *P. grandigclava* is not due to host selection but phylogeographic processes that occurred in the evolutionary history of this species.

Individuals collected from Northern Italy formed a separate clade ("clade A"), distinct from the haplotypes detected in Austria and the Czech Republic. Although there are too few individuals for a phylogeographic interpretation, the Italian Peninsula often harbours genotypes distinct from the rest of Europe (Hewitt, 1999). This is also the case for the scolytid *Pityogenes chalcographus* L. (Avtzis et al., 2008).

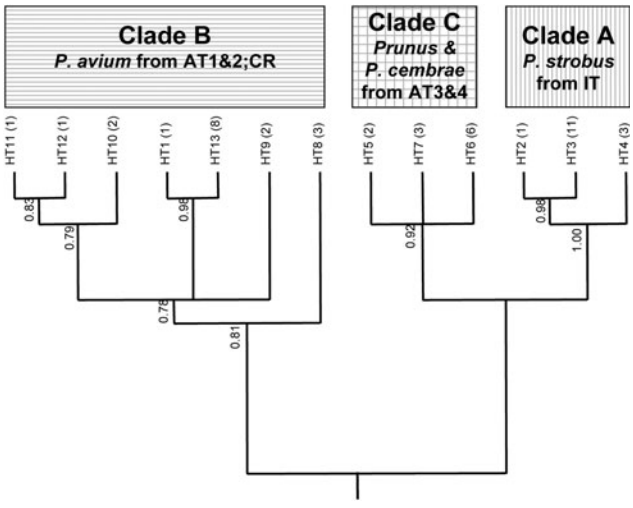

Fig. 1 The 50% majority rule consensus tree resulting from the Bayesian inference based on the analysis of a 579 bp long partial region of the mitochondrial COI gene of 47 *P. grandiclava* individuals. The Bayesian inference was calculated using the substitution models proposed by MrModeltest v2.1. Numbers above branches indicate Bayesian posterior probabilities (> 0.60). The number of individuals is given in parentheses.

TABLE 2. Analysis of Molecular Variance (AMOVA) among the populations of *P. grandiclava* assessed by regions and by host preference, as well as by clade assignment. *** $P < 0.001$.

		Source of variation	Variance components	% of variation
Grouping by region	[Italy] vs [Czech R] vs [Austria]	among groups	–0.03894Va	–0.45
		among populations/ within groups	6.27576Vb	72.01***
		within populations	2.47771Vc	28.43***
Grouping by host	[Conifers] vs [Broadleaf]	among groups	1.07353Va	12.01
		among populations/ within groups	5.38808Vb	60.27***
		within populations	2.47771Vc	27.72***
Grouping by clades	[clade A] vs [clade B] vs [clade C]	among groups	8.59735Va	90.83***
		among populations/ within groups	0.86765Vb	9.17***
		within populations	0.00000 Vc	0.00

This is a preliminary study and future analysis should concentrate on AFLP markers (Mock et al., 2007) as microsatellite loci seem to be less polymorphic in scolytid species than other insect orders (Arthofer et al., 2008). Although one can rarely collect *P. grandiclava* from both host species at the same location, more such populations need to be studied along with the biological aspects of host adaptation. This should include a test of the fitness and/or behavioural responses to each host, which would indicate the degree of differentiation between populations adapted to each host.

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