FOLIA ENTOMOLOGICA HUNGARICA
ROVARTANI KÖZLEMÉNYEK
Volume 68 2007 pp. 97–104.

Occurrence of the introduced Xylosandrus germanus (Blandford, 1894) in Hungary – a genetic evidence (Coleoptera: Scolytidae)

F. LAKATOS[1] & H. KAJIMURA[2]

[1]*University of West Hungary, School of Forestry, Institute of Sylviculture and Forest Protection, 9401 Sopron, Pf. 132, Hungary. E-mail: flakatos@emk.nyme.hu*
[2]*Nagoya University, Graduate School of Bioagricultural Sciences, Laboratory of Forest Protection, Chikusa, Nagoya 464-8601, Japan. E-mail: kajimura@agr.nagoya-u.ac.jp*

Abstract – In June 2005 *Xylosandrus germanus* (BLANDFORD, 1894), a new ambrosia beetle for Hungary was found close Nagymáté, County Baranya. The adults were excavated from freshly felled logs of *Quercus* and *Tilia*, and reared from these attacked trees in light eclector respectively. The prepared specimens are deposited at the Hungarian Natural History Museum (Coleoptera Collection) and at the Institute of Sylviculture and Forest Protection, University of West-Hungary. In its natural distribution *X. germanus* is considered as an important pest, while in Europe – where the species was introduced more than 60 years ago – no remarkable damage was reported. Genetic analyses – a comparison of the 400bp fragment of the mitochondrial COI gene – of Hungarian and German individuals were performed to confirm the species' status. The investigated populations can be considered identical (0.25% divergence, 2 haplotypes: GenBank accession numbers: HT-1 EF433438 and HT-2 EF433439). As outgroup for the analyses sequence data of *Xylosandrus mancus* (BLANDFORD, 1894) from the GenBank (AF187143) was used.

Key words – *Xylosandrus germanus*, new record, Hungary, mtDNA, COI.

INTRODUCTION

The investigation of the Hungarian scolytid fauna was rather extensive in the last decades. The last detailed description was published by ENDRŐDI (1959). The checklist of the Hungarian weevils (family Curculionidae) – where, regarding the latest taxonomic rating, also the former family of the

Scolytidae belongs to – was published eleven years ago by PODLUSSÁNY (1996). He listed altogether 105 bark and ambrosia beetles for Hungary. Some more detailed investigations were made on some forestry related, or damage causing species (LAKATOS 2006).

However, it doesn't mean, that no new arrivals were recorded for the last 50 years. Some recent examples are: *Xyleborus affinis* EICHHOFF, 1868, which were introduced to Hungary with Dracaena live stocks (TUSNÁDI & MERKL 1991); *Phloeosinus aubei* (PERRIS, 1855) and *P. thujae* (PERRIS, 1855) which expanded their natural Mediterranean distribution area to Hungary (RAKK & BÜRGÉS 1994); and *Ips amitinus* (EICHHOFF, 1871) and *Carphoborus minutus* (FABRICIUS, 1798), which were found first during detailed investigation of the Hungarian forests (LAKATOS 2006).

In 2005 we have found a further species. The importance of *Xylosandrus germanus* (BLANDFORD, 1894) is highlighted by the fact that both on his native distribution area (Asia) and in North-America (where it was introduced in the early 20th century) the species is considered as an important pest.

The aim of our investigations was (1) to confirm the presence of *X. germanus* in Hungary using morphological characters and genetic markers, and (2) to evaluate the importance and hazard of the species for the Hungarian forestry.

MATERIALS AND METHODS

Sample collection – Individuals of *X. germanus* were collected in Hungary (Nagymáté, Baranya county; N: 46° 11' 10.78" E: 17° 58' 35.56"). Altogether four beetles were excavated from felled *Quercus* and *Tilia* logs, on the 31 May 2005 by F. LAKATOS and H. KAJIMURA; and further 32 specimens were reared from the attacked logs in light eclectors at the Institute. The prepared specimens are deposited in the Coleoptera Collection of the Hungarian Natural History Museum (Budapest) and at the Institute of Sylviculture and Forest Protection, University of West Hungary (Sopron). Further individuals were received from South Germany (Baden-Württemberg), collected by TOBIAS BEIGEL (Table 1). Samples were placed into absolute ethanol and stored at –20 °C.

Molecular analysis – Insect DNA was extracted using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, USA) following the manufacturer's protocol. The extracted DNA was stored at 4 °C for up to 3 weeks, but for long term storage DNA was kept at –20 °C. The amplification of the DNA was carried out in 25 µl reactions containing 3.75 mM $MgCl_2$ 125 µM dNTPs (Sigma-Aldrich, USA), 0.5 µM of the primers "Dick" 5'ccaacaggaattaaaatttttagatgattagc-3' (position: 2410-2441) and "Pat" 5' tccattgcactaatctgccatatta -3' (position: 3014-3039) (LUNT *et al.* 1996) and 1U of Promega Taq. The amplifications were carried with an initial denaturation step of 3 min at 94 °C, which was followed by 35 cycles of

94 °C (30 sec), 48 °C (60 sec) and 68 °C (90 sec) and a final extension step at 68 °C (10 min). PCR was performed in an Eppendorf Mastercycler in 200 µl tubes. The sequence reactions were performed on an AB 3730XL sequencer at the University of Illinois, Chicago.

Statistical analysis – The mitochondrial DNA sequence alignment was performed by Clustal X (THOMPSON *et al.* 1997) using default setting. Distance analysis was performed by the Neighbor Joining NJ algorithm (SAITU & NEI 1987, STUDIER & KEPPLER 1988) as it is implemented in the MEGA version 3 (KUMAR *et al.* 2004). The distance matrix was calculated based on the Tamura-Nei substitution model (TAMURA & NEI 1993), while the robustness of the topology was tested by bootstrapping with 1000 repetitions (FELSENSTEIN 1985, 1988). For comparison also the GenBank entry of *Xylosandrus mancus* (BLANDFORD, 1898) (submitted by NORMARK *et al.*: AF187143) were taken.

RESULTS

Morphological comparison – The collected individuals (all females) were compared to individuals deposited at the Coleoptera Collection (Hungarian Natural History Museum, Budapest). All comparison materials originated from Germany, collected between 1953 and 1974. Both the antennal and elytral morphology were similar.

Genetical comparison – Five individuals from Hungary represent one haplotype (HT-1), while the three German individuals represent two haplotypes (HT-1 and HT-2) on the 397bp fragment of the COI gene (Table 1). The outgroup *X. mancus* showed a sequence divergence of 20.91% to the *X. germanus* populations (Table 2, Fig. 1). The vast majority of sequence variation observed occur on third codon positions (65 sites, 78.31%) followed by first (13 sites, 15.67%) and second codon positions (5 sites, 6.02%).

Both haplotypes found (HT-1 and HT2) were submitted to the GenBank under the accession numbers HT-1 EF433438 and HT-2 EF433439.

Table 1. Collection sites and haplotypes of investigated *Xylosandrus germanus* samples

Country	Area	Individual/ Abbreviation	Haplotype	GenBank identifier
Hungary	Nagymáté	HU-1	HT-1	EF433438
		HU-2	HT-1	
		HU-3	HT-1	
		HU-4	HT-1	
		HU-5	HT-1	
Germany	Baden-Württemberg	DE-1	HT-1	
		DE-5	HT-2	EF433439
		DE-6	HT-2	

Table 2. Sequences and mutation sites of investigated *Xylosandrus germanus* haplotypes and *Xylosandrus mancus* sequences from GenBank

```
XGHU-1_HT-1 AAA ATT TTT AGA TGA TTA GCA ACA TAC CAC GGA ACA CAA ATC TCT GCC AGA CCT AGA TCT CTG TGA GCT TTA GGA TTT
XGDE-5_HT-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X_mancus    ... ... ... ..T ... ... ... ..T ... ... ... ... ... ... ..A ATA .C. ..A ... ... T.A ... ..C C.T ..T ...

XGHU-1_HT-1 TTA TTC CTA TTC ACC CTA GGG GGA TTA ACA GGA GTT GTG TTA GCC AAC TCA TCT CTT GAT ATC ATC CTA CAT GAC ACA
XGDE-5_HT-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X_mancus    C.. ... ..G ... ..T A.. ..A ... ... ... ..T ..A A.T ... ..A ..T ... ... ... ... ..T ..T ... ... ..T ...

XGHU-1_HT-1 TAT TAT GTT GTT GCA CAT TTC CAC TAT GTT CTA TCC ATA GGG GCA GTA TTC GCC ATT ATA GCC GGG CTT GTT CAA TGA
XGDE-5_HT-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X_mancus    ..C ... ..A ..A ..C ... ..T ... ... ..C ... ..A ... ..A ... ... ..T ..T ... ... ..A ..A A.C ..C ... ...

XGHU-1_HT-1 TTC CCC TTA TTC ACT GGA CTT ACA CTA AAT AAT AAA TAT TTA AAA ACA CAA TTC ATC TCT ATA TTT ATT GGG GTA AAC
XGDE-5_HT-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X_mancus    ..T ..A C.. ..T ..A ..G ..A ... ..T ..C ... ... ..C ... ... ... ... ..T ... ... ... ... ... ... ... ...

XGHU-1_HT-1 ATA ACT TTC TTT CCC CAA CAC TTT CTA GGA TTA AGA GGA ATA CCA CGA CGA TAC TCA GAT TAT CCA GAT GCA TAC ATC
XGDE-5_HT-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X_mancus    ... ... ... ..C ..A ..G ... ..C ... ..G ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... ..T ..T

XGHU-1_HT-1 ATA TGA AAC ATC ATT TCC TCA ATT GGC AGA CTA ATC TCA TTA ATA AGA ATT TTA TTT TTC ATT TTT ATT ATT TGA GAA
XGDE-5_HT-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X_mancus    ... ..G ... ..T ..C ... ... ... ..A ... A.. ..T ... C.G ... ... ... A.. ... ... ..C ... ..C ..C ... ...

XGHU-1_HT-1 AGA TTT TCT GTT AAA CGA TTA GCT ATT AC
XGDE-5_HT-2 ... ... .T. ... ... ... ... ... ... ..
X_mancus    GCC ..: .CA ..C ... ... CC. ... ... ..
```

DISCUSSION

Fifty two known species belong to the genus *Xylosandrus* REITTER, 1913. Most of them are native to the tropical and subtropical Southeast Asia. Five species can be found in North America (but four of them are introduced) and only one (also introduced), *X. germanus* in Europe (WOOD & BRIGHT 1992). The closest genus is *Xyleborus* EICHHOFF, 1864, which is shown also by several *Xylosandrus* species which were described first as *Xyleborus* species (also *Xylosandrus germanus*). Some species of the genus, e.g. *Xylosandrus mutilatus* (BLANDFORD, 1894), are investigated more in details (KAJIMURA & HIJII 1992, 1994), but most of them are poorly known.

The detailed description of body characters are presented by PFEFFER (1995) and WOOD & BRIGHT (1992). The main differences between male and female beetles are typical as usual for all Xyleborini. The flying female is 2.0–2.3 mm, while the flightless male is spheroid and 1.0–1.8 mm large. Both the body characters and gallery structures are similar to *Xyleborus dispar* (FABRICIUS, 1792), which is one of the most common Xyleborini in Hungary, but the body size is smaller by *X. germanus*. The typical gallery is a so called "family hole", where all the adults and progeny are feeding on the ambrosia fungi. The most typical features are the toothpick like sawdust protruding from the entry holes.

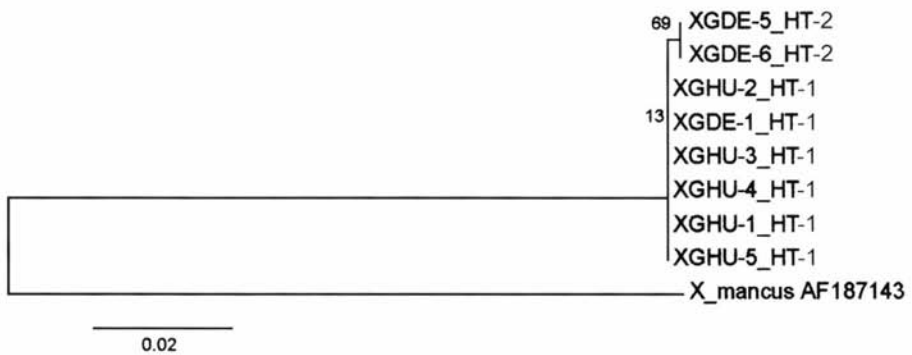

Fig. 1. Neighbour joining tree of the investigated *Xylosandrus germanus* (BLANDFORD, 1894) individuals and *Xylosandrus mancus* (BLANDFORD, 1894) sequences as an outgroup (Tamura-Nei parameter) available in the GenBank. Bootstrap values are given at nodes

The species was described from Japan. The original distribution area is East and Southeast Asia (Japan, Korea and the eastern part of China). It was introduced to the USA (1932), where it was discovered in imported wine stocks in greenhouses. The species escaped and became an important pest on different woody plants (ATKINSON *et al.* 1990). In Europe it was first recorded after the WWII in Germany (GROSCHKE 1953). The detailed investigations figured out, that the species might be introduced with wood pieces imported from Japan to South-Germany already at the beginning of the 20 century. The species expanded its distribution area slowly to natural beach (*Fagus*) and oak (*Quercus*) stands. The present distribution area includes France, Belgium (BRUGE 1995), Germany (GROSCHKE 1953), Switzerland (MAKSYMOV 1987, GRAF & MANSER 1996), Italy (FACCOLI 2000), Austria and Russia (MANDELSHTAM 2000). Many other countries must belong to the present distribution are too, but because of the lack of adequate information no details are available.

X. germanus is highly polyphagous. In its native distribution area a great variety of broadleaved and evergreen wood and shrub species can be affected (e.g. tea shrub). The host range in the introduced areas (Europe and North America) also includes many plant species, like: *Quercus*, *Fagus*, *Acer*, *Alnus*, *Betula*, *Buxus*, *Carpinus*, *Castanea*, *Corylus*, *Ficus*, *Juglans*, *Robinia* (!), *Ulmus*, *Picea*, *Pinus* and *Abies* (POSTNER 1974).

The judgement of *X. germanus* is still controversial. The high number of possible host plants and the reported damages especially in the USA – but also in the native areas – assume a high risk also for the European forest ecosystems. However, none of the investigations supported this assumption. The species is presented in many countries for many years, but until now no considerable damage was reported from Europe. It is considered everywhere as a secondary pest, which can feed only in freshly felled logs and stumps. We have excavated also the first individuals from freshly felled oak and linden logs, and reared from attacked tree parts at the Institute of Sylviculture and Forest Protection.

Genetic analyses – the comparison of the 400bp fragment of the mitochondrial COI gene – also confirmed the high similarity of Hungarian and German individuals, e.g. *Xylosandrus germanus* was found. Bootstrap values calculated indicate the extension of both the length of COI fragment and the number of individuals. The method should be used for species identification also in other insect taxa.

*

Acknowledgements – We would like to thank TOBIAS BEIGEL (Forest Research Institute of Germany, Baden-Württemberg) who provided us specimens for the genetic analyses. Special thanks are due to ATTILA PODLUSSÁNY, and OTTÓ MERKL (Hungarian Natural History Museum, Coleoptera Collection, Budapest) for their help during the identification of the species, and to two anonymous reviewers for their valuable comments on the manuscript. The stay of HISASHI KAJIMURA in Hungary was supported by the international exchange grant HAS-JSPS. A part of this study was supported by INAMORI Foundation (2005) and by the Hungarian Scientific Research Fund (OTKA K-63123).

REFERENCES

ATKINSON, T. H., RABAGLIA, R. J. & BRIGHT, D. E. 1990: Newly detected species of Xyleborus (Coleoptera: Scolytidae) with a revised key to species in eastern North America. – *Canadian Entomologist* **122**: 93–104.

BRUGE, H. 1995: Xylosandrus germanus (Blandford, 1894) (Coleoptera Scolytidae). – *Bulletin & Annales de la Société royale belge d'Entomologie* **131**: 249–264.

ENDRŐDI, S. 1959: Szúbogarak – Scolytidae. – In: *Magyarország Állatvilága* (*Fauna Hungariae*), *10, 9.* Akadémiai Kiadó, Budapest, 96 pp.

FACCOLI, M. 2000: Bio-ecology of bark beetles *Ips typographus* (L.) and species recently affecting the Italian forests. Part III. Notes about some bark beetle species new to Italy. – *Bollettino dell'Istituto di Entomologia "Guido Grandi" della Università degli Studi di Bologna* **54**: 77–90.

FELSENSTEIN, J. 1985: Confidence limits on phylogenies: An approach using the bootstrap. – *Evolution* **39**: 783–791.

FELSENSTEIN, J. 1988: Phylogenies and quantitative characters. – *Annual Review of Ecology and Systematics* **19**: 445–471.

GRAF, E. & MANSER, P. 1996: Der Schwarze Nutzholzborkenkäfer Xylosandrus germanus in der Schweiz. – *Holz Zentralblatt* **122**: 454–456.

GROSCHKE, F. 1953: Der Schwarze Nutzholzborkenkäfer, eine neue Gefahr für Forstwirtschaft, Obst- und Wienbau. – *Anzeiger für Schädlingskunde* **26**: 81–84.

KAJIMURA, H. & HIJII, N. 1992: Dynamics of the fungal symbionts in the gallery system and the mycangia of the ambrosia beetle, Xylosandrus mutilatus (Blandford) (Coleoptera: Scolytidae) in relation to its life history. – *Ecological Research* **7**: 107–117.

KAJIMURA, H. & HIJII, N. 1994: Reproduction and resource utilization of the ambrosia beetle, Xylosandrus mutilatus, in field and experimental populations. – *Entomologia Experimentalis et Applicata* **71**: 121–132.

KUMAR, S., TAMURA, K. & NEI, M. 2004: MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. – *Briefings in Bioinformatics* **5**: 150–163.

LAKATOS, F. 2006: Fenyőállományokban végrehajtott egészségügyi termelések szerepe védett és/vagy veszélyeztetett fában és kéregben költő bogárfajok esetén. (The impact of sanitary cutting on the protected and/or endangered bark and wood boring beetles in coniferous forests (Coleoptera).) – *Természetvédelmi közlemények*: **12**: 123–131.

LUNT, D., ZHANG, D. X., SZYMURA, J. M. & HEWITT, G. M. 1996: The insect COI gene: Evolutionary patterns and conserved primers for phylogenetic studies. – *Insect Molecular Biology* **5**: 153–165.

MAKSYMOV, J. K. 1987: Erstmaliger Massenbefall des schwarzen Nutzholzborkenkäfers, Xylosandrus germanus Blandf., in der Schweiz. – *Schweizerische Zeitschrift für Forstwesen* **138**: 215–227.

MANDELSHTAM, M. J. 2000: New synonymy and new records of Palearctic Scolytidae (Coleoptera). – *Zoosystematica Rossica* **9**(1): 203–204.

PFEFFER, A. 1995: *Zentral- und westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae)*. – Pro Entomologia, c/o Naturhistorisches Museum, Basel, 69 pp.

PODLUSSÁNY, A. 1996: Magyarország ormányosalkatú bogarainak fajlistája (Coleoptera: Curculionoidea). (A check-list of the superfamily Curculionoidea (Coleoptera) of Hungary.) – *Folia entomologica hungarica* **57**: 197–225.

POSTNER, M. 1974: Scolytidae (Ipidae), Borkenkäfer. – In: SCHWENKE, W. (ed.): *Die Forstschädlinge Europas. Vol. 2.* Paul Parey Verlag, Hamburg, Berlin, pp. 334–482.

RAKK, ZS. & BÜRGÉS, GY. 1994: Pusztít a borókaszú! (On the damage of the juniperus bark beetle (Coleoptera, Scolytidae: Phloeosinus aubei Perris).) – *Növényvédelem* **30**: 7–10.

SAITU, N. & NEI, M. 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. – *Molecular Biology and Evolution* **4**: 406–425.

STUDIER, J. A. & KEPPLER, K. L. 1988: A note on the neighbor-joining algorithm of Saitou and Nei. – *Molecular Biology and Evolution* 5: 729–731.T

AMURA, K. & NEI, M. 1993: Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. – *Molecular Biology and Evolution* **10**: 512–526.

THOMPSON, J. D., GIBSON, T .J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D. G. 1997: The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. – *Nucleic Acids Research* **24**: 4876–4882.

TUSNÁDI, CS. K. & MERKL, O. 1991: A dracénaszú (Xyleborus affinis Eichhoff; Coleoptera: Scolytidae) előfordulása Dracaena fragrans 'Massangeana' törzsekben. (The occurrence of Xyleborus affinis Eichhoff (Col.: Scolytidae) in Hungary in imported Dracaena fragrans stems.) – *Növényvédelem* **27**: 296–302.

WOOD, S. L. & BRIGHT, D. E. 1992: A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: taxonomic index. – *Great Basin Naturalist Memoirs* **13**: 1–1553.