Accepted 05 June 2004
JZS doi: 10.1111/j.1439-0469.2004.00279.x

[1]*Dipartimento Biologia Evoluzionistica Sperimentale, Bologna, Italy;* [2]*Istituto Sperimentale per la Zoologia Agraria, Sezione di Entomologia forestale, Via di Lanciola 12/A, Firenze, Italy*

Taxonomy and phylogeny of European *Monochamus* species: first molecular and karyological data

M. Cesari[1], O. Marescalchi[1], V. Francardi[2] and B. Mantovani[1]

Abstract

The worldwide distributed genus *Monochamus* Megerle, 1821 (Coleoptera Cerambicydae) comprises beetles that may become pests of economic importance in conifer stands in the Nearctic and Palearctic Regions. Besides direct damage due to the larval tunnelling habits, they have also been recognized as main vectors of the phytoparasitic nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) (Nematoda Aphelenchoididae). We analysed the complete mitochondrial cytochrome oxidase I gene and a fragment of the small subunit RNA gene sequences (1536 base pairs) in the five European species. These are: *Monochamus galloprovincialis* (Olivier, 1795), morphologically distinguished in two subspecies *M. galloprovincialis galloprovincialis* (Olivier, 1795) and *M. galloprovincialis pistor* (Germar, 1818); *Monochamus sutor* (Linneus 1758); *Monochamus saltuarius* (Gebler 1830); *Monochamus sartor* (Fabricius, 1787) and *Monochamus urussovi* (Fischer, 1806). For appropriate comparisons, also the Asiatic *Monochamus alternatus* Hope, 1842 and a Japanese *M. saltuarius* sample have been analysed. Both genes show an absolute identity between the two subspecies of *M. galloprovincialis* and a strong affinity between *M. sartor* and *M. urussovi*: the morphological subdivisions of the former taxon in two subspecies and of the latter in two entities of specific level are therefore not supported genetically. On the other hand, the Italian and the Japanese samples of *M. saltuarius* always cluster together in all trees, and for the remaining taxa, no doubt about their rank of specific differentiation emerges from present analyses. From a phyletic point of view, tree topology indicates the Japanese *M. alternatus* as the most differentiated taxon and the Euroasiatic *M. saltuarius* as basal to all other strictly European entities. Chromosome analyses show that the diploid autosomal complement ranges from 18 in *M. saltuarius* to 20 in *M. galloprovincialis*, and 22 in *M. sartor*, but a $XX–Xy_p$ sex determining system is shared by all analysed taxa. The *M. saltuarius* karyotype appears as the most primitive from which the others may be derived through Robertsonian fissions. Karyological data therefore agree with molecular analyses in indicating a basal position of Euroasiatic *M. saltuarius* with respect to the group of European *Monochamus* taxa; among these, *M. galloprovincialis* and *M. sartor* represent two clearly diverging evolutionary units. Furthermore, karyotype analyses substantiate molecular conclusions about the identity between *M. galloprovincialis galloprovincialis* and *M. galloprovincialis pistor*.

Key words: cytochrome oxidase II – 12S – gene trees – parsimony – karyotype

Introduction

The genus *Monochamus* Megerle, 1821 (Coleoptera Cerambicydae) comprises about 163 species, with a worldwide distribution and different trophic specializations (Hellrigl 1971; Goidanich 1972). In the Nearctic and in the Palaearctic regions, these beetles may become pests of economic importance in conifer stands, especially when, given appropriate environmental conditions, their population level increases enormously (Dominik 1982; Shao et al. 1988; Vallentgoed 1991; Morewood et al. 2002). *Monochamus* species are secondary xylophagous insects: adults feed on young twigs of healthy trees and lay eggs in weakening or dying plants, where larvae feed on phloem and cambium in the subcortical zone. On the whole, *Monochamus* taxa play therefore an important role in the trophic network and energy flux of forest ecosystems.

The importance of *Monochamus* species increased dramatically after the beginning of the last century when they were recognized as the main vectors of the phytoparasitic nematode *Bursaphelencus xylophilus* (Steiner & Buhrer, 1934) (Nematoda Aphelenchoididae) considered an A1 organism by the European and Mediterranean organization for the protection of plants (Smith et al. 1992). This nematode, native of Northern America, is responsible for extensive pine wilt diseases throughout the Far East regions, particularly in Japan, where it has been accidentaly introduced and spread by *M. alternatus* Hope, 1842 (Evans et al. 1996). Recently, the nematode has been recorded in Portugal, where *M. galloprovincialis* (Olivier, 1795) is assumed to be its main vector (Mota et al. 1999; Mota 2002). *Monochamus* adults passively carry the nematode and transmit it through the feeding wounds on healthy pines and, secondarily, through cortical oviposition scars made by females on diseased plants (Linit 1987; Evans et al. 1996).

Monochamus taxonomy is so far based on several morphological features such as body length and colour, elytral shape, distribution of microsculpture, extent and colour of the pubescence on the pronotum, elytra and antennae. However, a consistent variability in phenotypic characters leads to taxonomic uncertainties as in the case of *M. galloprovincialis* subspecies (Müller 1949–1953; Hellrigl 1971; Sama 1988, 2002) or inside the *M. urussovi* (Fischer, 1806) species (Grodnitskii et al. 1992). In particular, *M. urussovi* and *M. sartor* (Fabricius, 1787) are considered vicariant taxa (Hellrigl 1971), but for both entities a synonymy with *M. rosenmuelleri* (Cederhjelm, 1798) has been independently put forward (Danilevsky 2003).

Beside body morphology, little information exists on *Monochamus*, so that we started, through molecular and karyological approaches, the characterization of these beetles, with the analyses of the five European species: *M. galloprovincialis*, with its two subspecies *M. galloprovincialis galloprovincialis* (Olivier, 1795) and *M. galloprovincialis pistor* (Germar, 1818); *M. sutor* (Linneus, 1758); *M. saltuarius* (Gebler, 1830); *M. sartor* and *M. urussovi*. Also a Japanese sample of *M. saltuarius* and specimens of the Asiatic *M. alternatus* were analysed (Table 1). We sequenced the complete mitochondrial cytochrome oxidase I (COI) gene and a fragment of the small subunit RNA gene (12S), for a total of

Table 1. Distribution and host plants for analysed taxa

Species	Host plants	Range
M. g. galloprovincialis	*Pinus pinaster*, *Pinus nigra*, *Pinus halepensis*, *Pinus sylvestris*, *Pinus insignis*, *Pinus pinea*	Europe, Siberia, North Africa, Asia Minor
M. g. pistor	*Pinus sylvestris*, *Pinus nigra*, *Picea abies*	Central-eastern Europe (Alps included)
M. sutor	*Picea abies*, *Pinus sylvestris*, *Pinus mugo*, *Pinus nigra*, *Abies alba*, *Larix* spp.	Western (Pyrenees), Middle (Alps included) and North-eastern Europe, Balcanic Peninsula (Romania, Bulgaria), Georgia, Siberia, China, Northern Mongolia, Japan, Korea, Sachalin
M. saltuarius	*Picea abies*, *Pinus sylvestris*, *Pinus nigra*, *Larix* spp., *Abies alba*	Middle (Alps included) and Eastern Europe, Siberia, China, Korea, Japan, Sachalin
M. sartor	*Picea abies*, *Pinus sylvestris*, *Pinus cembra*, *Pinus mugo*, *Abies alba*	Middle (Alps included) and Eastern Europe (Western Carpathians), Balcanic Peninsula, Ukraine
M. urussovii	*Picea abies*, *Abies sibirica*, *Abies alba, Pinus* spp.	Northern and Eastern Europe, Siberia, China, Japan
M. alternatus	*Pinus densiflora*, *Pinus thunbergii*, *Cedrus spp*., *Abies* spp., *Picea* spp., *Larix* spp.	Asia (Japan, Korea, Taiwan, Laos, China)

1536 base pairs. These mitochondrial genes have been frequently used to address phylogenetic relationships in insects. Especially in Coleoptera, the COI gene region has proven very useful to resolve phylogenetic relationships at the generic level and below (Funk et al. 1995; Howland and Hewitt 1995), while at the family and higher levels it rarely gives satisfactory resolution (Brown et al. 1994; Miura et al. 1998) and it is often unreliable (Dowton and Austin 1997; Mardulyn and Whitfield 1999) owing to its high variability.

Coleoptera represents an interesting group also from a karyological point of view and, so far, more than 3000 beetle species have been cytogenetically studied (Smith and Virkki 1978; Juan and Petitpierre 1991; Petitpierre 1996). They show a very wide range of chromosome numbers varying from $2n = 4$ (male meioformula = 1 + XY) in the click beetle *Chalcolepidius zonatus* Eschscholtz, 1829 (Ferreira et al. 1984) to $2n = 69$ (male meioformula = 34 + X) in the ground beetle *Ditomus capito* Audinet-Serville, 1821 (Serrano 1981). In addition, sex determining systems are surprisingly variable in having one, two or more sex-chromosomes involved, either non-chiasmatic or chiasmatic (see Smith and Virkki 1978, for a review). The karyotype and meiotic dynamics of the genus *Monochamus* have been so far studied only in the American taxa *M. notatus* Drury, 1773, *M. marmorator* Kirby, 1837 and the *M. scutellatus* Say, 1824 – *oregonensis* LeConte, 1873 complex (Smith 1950, 1953; Lanier and Raske 1970). In these taxa a stability of the autosomal number ($2n = 18$) and a great variation in number and size of sex chromosomes (XXY_p, XY_p, Xy_p, etc.) was observed. Since living specimens are necessary for chromosomal analysis, it has been possible to study only samples of *M. saltuarius*, *M. sartor*, and of *M. galloprovincialis* subspecies.

This paper aims to the karyological and molecular characterization of the European species of *Monochamus*, and in particular to evaluate the differentiation level of *M. galloprovincialis* subspecies and of the relationships between *M. sartor* and *M. urussovi*.

Materials and methods

Morphologically determined beetles were frozen or alcohol-preserved and then used as source material for DNA extraction and amplification of the COI and 12S mitochondrial genes through polymerase chain reaction (PCR). Sample information are given in Table 2.

Genomic DNA was prepared from single beetle heads following the method described in Preiss et al. (1988). PCR amplification was performed in 50 µl reactions using the Invitrogen kit, with *Taq* DNA polymerase recombinant. Thermal cycling was done in a GeneAmp PCR System 2400 (Applied Biosystems, Foster City, California, USA)

Table 2. Taxa, sample collecting place, acronyms and scored haplotypes

Taxon	Collecting place	Country	Acronym	Haplotypes 12S	Haplotypes COI
M. g. galloprovincialis	Montefalcone-Pisa	Italy	GGmo1	1	1
M. g. galloprovincialis	Montefalcone-Pisa	Italy	GGmo2	1	1
M. g. pistor	Mules-Bolzano	Italy	GPmu1	1	1
M. g. pistor	Villa Santina-Udine	Italy	GPvs1	1	1
M. sartor	Cima Gogna-Belluno	Italy	SAcg1	2	2
M. sartor	Mules-Bolzano	Italy	SAmu1	2	2
M. sartor	Lorenzago-Belluno	Italy	SAlo1	2	3
M. sutor	Val di Genova-Trento	Italy	SUvg1	3	4
M. sutor	Val di Genova-Trento	Italy	SUvg2	3	5
M. saltuarius	Mules-Bolzano	Italy	SLmu1	4	6
M. saltuarius	Mules-Bolzano	Italy	SLmu2	4	6
M. saltuarius	Takano Town-Hiroshima	Japan	SLjp1	5	7
M. saltuarius	Takano Town-Hiroshima	Japan	SLjp2	5	7
M. urussovii	Bialowieza Primeval Forest	Poland	URpl1	6	8
M. urussovii	Bialowieza Primeval Forest	Poland	URpl2	6	8
M. alternatus	Futsu city-Chiba	Japan	ALjp1	7	9
M. alternatus	Futsu city-Chiba	Japan	ALjp2	7	9

with the following cycle: denaturation at 94°C for 30 s; annealing at 48°C for 30 s and extension at 72°C for 30 s. The amplified products were purified with the Concert™ Rapid Purification System kit (Invitrogen, Carlsbad, California, USA) and strands were sequenced with the Big Dye Terminator sequencing kit (Applied Biosystems) in an ABI PRISM 310 Genetic Analyzer. The primers for PCR amplifications were: mtD-35 (AAG AGC GAC GGG CGA TGT GT)/mtD-36 (AAA CTA GGA TTA GAT ACC CTA TTA T) for the 12S gene, and mtD-7 (GGA TCA CCT GAT ATA GCA TTC CC)/ mtD-12 (TCC AAT GCA CTA ATC TGC CAT ATT A) for the COI gene. In addition, the mtD-10 (TTG ATT TTT TGG TCA TCC AGA AGT) primer was used in sequencing reactions for the COI gene. Primers were derived from Simon et al. (1994).

Sequences were aligned using the Clustal algorithm of the Sequence Navigator program (version 1.0.2b3, Applied Biosystems) and checked by eye. The nucleotide sequences of the analysed *Monochamus* specimens were submitted into GenBank under the accession numbers AY260835-AY260845, AY264403 (COI) and AY258053-AY258063 (12S).

For the COI gene, translation to amminoacids was obtained using the *Drosophila* mitochondrial genetic code (De Bruijn 1983; Jermin and Crozier 1994).

Nucleotide and amminoacid composition, average codon frequencies and relative synonimous codon usage (RSCU), as well as distance matrices following the Kimura two-parameter method were performed using MEGA version 2.1 (Kumar et al. 2001).

Neighbor Joining, maximum parsimony (MP) and maximum-likelihood phylogenetic analysis were performed with PAUP* (version 4.0b10; Swofford 2001). Bootstrap analyses were performed with 500, 500 and 100 replicates, respectively. For maximum-likelihood analysis, the best substitution models (F81 + G for the 12S gene, GTR + G for the COI gene and TVM + G for combined sequences) were estimated using Model Test 3 (version 3.06; Posada and Crandall 1998), to determine the settings corresponding to R matrix, base frequencies, proportion of invariable sites and value of gamma shape parameter.

Phaea maryannae Chemsak, 1977 (Coleoptera Cerambycidae; GenBank A.N.: AF267467) and *Oreina speciosa* L., 1758 (Coleoptera Chrysomelidae; GenBank A.N.: AF097074) were used as outgroups for the COI gene and the 12S gene, respectively.

Mitotic chromosome preparations of *M. galloprovincialis pistor* were made from cerebral ganglia of young larvae. Testes and ovaries from adults were used to obtain mitotic or meiotic chromosomes of *M. saltuarius*, *M. galloprovincialis galloprovincialis* and *M. sartor*. To increase the number of metaphases and the sharp centromere localization, anaesthetized animals were injected with a 0.05% solution of colchicine 1 h before dissection. The tissues were treated with a hypotonic solution of Na-citrate 1% for 20 min, fixed in 3:1 methanol–acetic acid for 30 min, dissociated on a clean slide with acetic acid 60% and air dried. Slides were stained with Giemsa solution 3% in phosphate buffer at pH 7 for 20 min. Chromosomes were classified according to the criteria and terms suggested by Levan et al. (1964). Karyotypes were constructed by arranging homologous chromosomes in order of decreasing size.

Results

Molecular analyses

The sequence analysis of the 12S gene covered 349 bp, corresponding to base number 14220–14557 of the complete mitochondrial genome of *Tribolium castaneum* Herbst, 1797 (Coleoptera Tenebrionidae; Genbank A.N.: AJ312413). The seven haplotypes scored differ by 2–18 substitutions. Each species shows a single haplotype, with the only exception of Italian and Japanese samples of *M. saltuarius*, which have different haplotypes. Moreover, haplotype 1 is shared by the two *M. galloprovincialis* subspecies. Seventeen of the 68 variable sites observed are parsimony informative. Overall proportion of A–T is 80,3%. Kimura-2-parameter distances range from 0.0058 (GGmo1 versus SUvg1) to 0.0544 (SLmu1 versus SAcg1/SUvg1/URpl2).

For the COI gene, we obtained 1185 bp: no insertions or deletions were found in this region. This segment corresponds to base number 3449–4617 of the *Pyrocoelia rufa* Olivier, 1886 (Coleoptera Lampyridae) complete mitochondrial genome (Genbank A.N.: NC_003970). The nine haplotypes scored differ by 1–91 substitutions. *M. galloprovincialis*, *M. urussovi* and *M. alternatus* show only one species specific haplotype, while two haplotypes are present in each of the other species. Again, the same haplotype (haplotype 1) is shared by the two *M. galloprovincialis* subspecies. The total number of variable sites is 262, whereas only 35 are parsimony informative. Overall proportion of A-T content is lower with respect to the 12S gene, being at 69.9%; even lower values are observed at the first and second codon positions (respectively 56.4% and 59.9%). The A-T content increases at 93.4% at the third codon position. Kimura-2-parameter distances range from 0.0008 (SAcg1 versus SAlo1) to 0.0815 (SLmu1 versus SUvg1).

Inferred aminoacid sequences differ by 2 to 7 aminoacid substitutions; these are mainly non-conservative replacements. P-distance values range from 0.0051 to 0.0178. The tree scored on aminoacid sequences is absolutely polytomic and it has not been taken into account.

Neighbour Joining and maximum parsimony trees obtained on the 12S gene (Fig. 1a) do agree in showing the presence of a tritomy, with the following groups: (a) the *M. alternatus* sample; (b) the *M. saltuarius* cluster; (c) the rest of scored haplotypes. The latter group is further subdivided in a cluster comprising *M. sartor* and *M. urussovi* samples, and another one with *M. galloprovincialis* and *M. sutor* haplotypes. All nodes are supported with fairly high bootstrap values. In the maximum likelihood tree (Fig. 1b) the third cluster collapses, generating two new groups.

For the COI gene, all trees agree (Fig. 1c; Neighbor Joining values are not shown, being available from the authors). The *M. alternatus* haplotype is basal to a dichotomy between *M. saltuarius* and all other taxa. The latter cluster shows a tritomy among *M. galloprovincialis*, a group encompassing *M. sartor* and *M. urussovi* haplotypes, and *M. sutor* haplotypes. All clusters are supported with high bootstrap values.

On a combined data set, Neighbor Joining and maximum parsimony topology (Fig. 1d) agrees with the COI gene in the basal positioning of *M. alternatus* haplotype and in a higher affinity between *M. galloprovincialis* and *M. sutor*, as evidenced in the 12S analyses. Every group is supported by high bootstrap values. The maximum likelihood tree (not shown) agrees with the described topology, with the exception of the basal position of *M. alternatus* losing bootstrap support.

Karyological analyses

Monochamus saltuarius

Female chromosome number is $2n = 20$ (Fig. 2a). The first chromosome pair is submetacentric and markedly larger than the others which are all distinctly dibrachial (pairs 3, 4, 5, 6, 7 and 9: metacentric; pairs 2 and 8: submetacentrics); the smallest pair (10) reaches a dot-like shape. No male mitotic plates were observed. Male meiosis appears quite normal (Fig. 2b), autosomal pairing is invariably complete and no heteromorphic bivalents are observed; sex chromosomes, paired in the form of a 'parachute', are easily identified (meioformula 9 + Xy_p). The size of sexual bivalent suggests that heterochromosomes are the smallest elements of the complement.

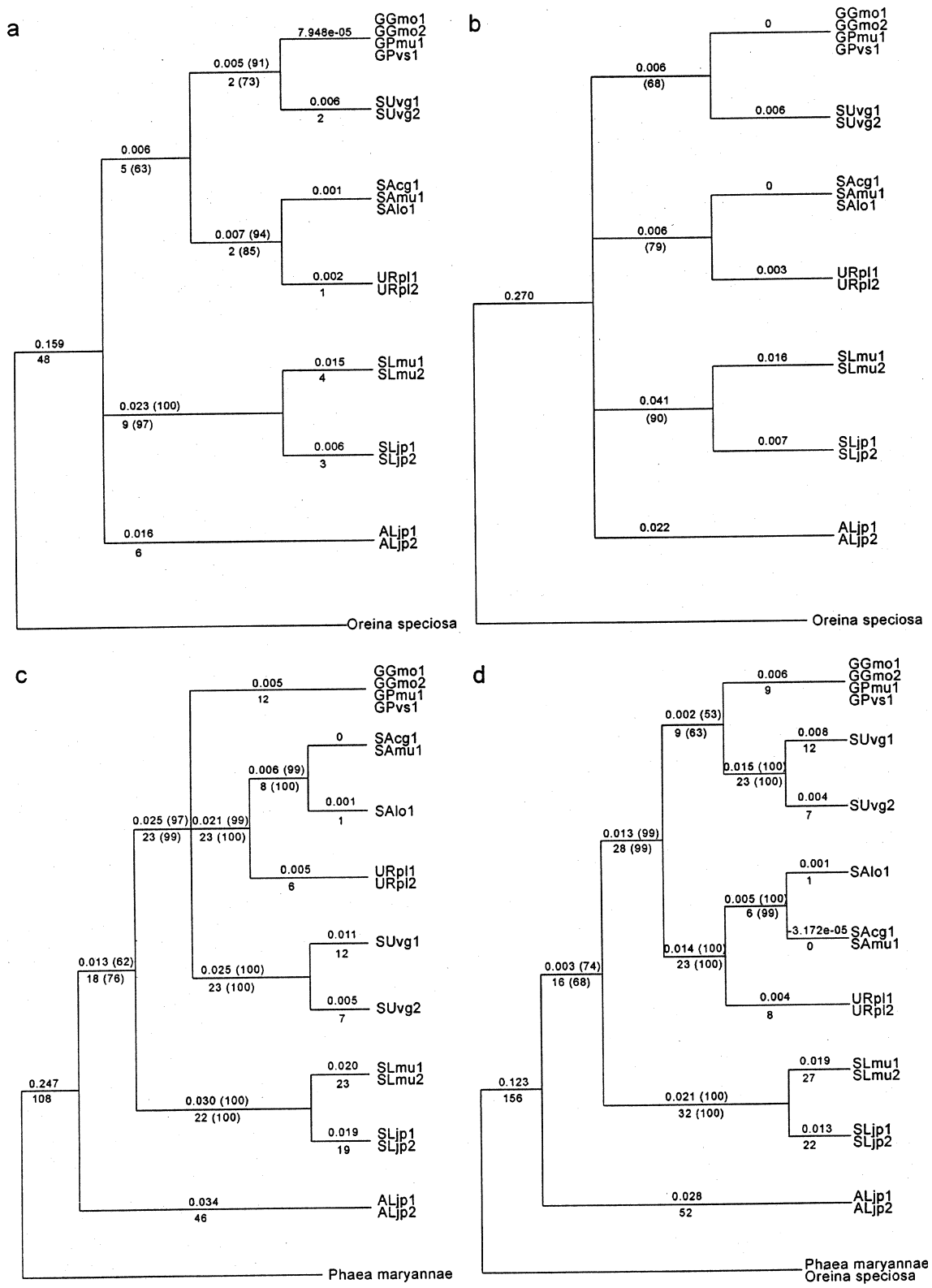

Fig. 1. Trees obtained on 12S haplotypes (a, b), COI haplotypes (c) and combined data sets (d). Elaborations as follows: (a) Neighbor Joining combined with maximum parsimony (consistency index: 0.902, retention index: 0.680, rescaled consistency index: 0.614); (b) maximum likelihood (−ln L likelihood = 776.366); (c) maximum likelihood (−ln L likelihood = 3233.593) combined with maximum parsimony (consistency index: 0.852, retention index: 0.798, rescaled consistency index: 0.679); (d) Neighbor Joining combined with maximum parsimony (consistency index: 0.872, retention index: 0.761, rescaled consistency index: 0.663). In (a) and (d) values above branches indicate distance values, while numbers below branches represent mutational steps; in (b) and (c) numbers above branches indicate substitution/site values; in (c) numbers below branches indicate mutational steps. Numbers in parentheses indicate bootstrap values. Acronyms as in Table 2

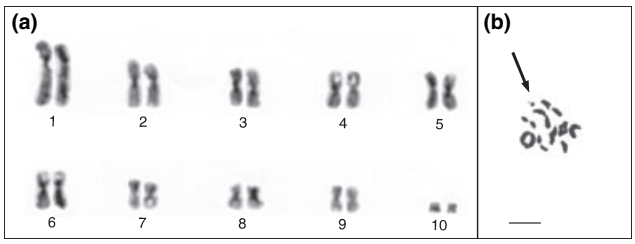

Fig. 2. *Monochamus saltuarius*: (a) Giemsa stained female karyotype; (b) male meiotic metaphase I with nine autosomal bivalents; sex chromosomes are associated in the 'parachute' configuration (arrow). Bar represents 5 μm

Monochamus galloprovincialis galloprovincialis
The diploid number based on female mitoses is $2n = 22$ (Fig. 3a). Its karyotype comprises six pairs of metacentrics (1, 3, 4, 5, 7 and 10), two pairs of submetacentrics (6 and 9) and three pairs of subacrocentrics (2, 8 and 11). The chromosome number observed in female mitotic plates was confirmed in male mitotic and meiotic metaphases (Fig. 3b). Autosomes form regular associations and the sexual bivalent in the 'parachute' configuration is clearly distinguishable by its heteromorphic character (male meioformula 10 + Xy_p). As in the preceding karyotype the sexual bivalent is the smallest.

Monochamus galloprovincialis pistor
Mitotic chromosome number is $2n = 22$; owing to the lack of meiotic plates, no sex pair could be directly ascertained. The karyotype shows six pairs of metacentrics (1, 3, 4, 5, 7 and 10), two pairs of submetacentrics (6 and 9) and two pairs of subacrocentrics (2 and 8). Chromosomes of pair 11 are strongly heteromorphic; in some plates, clues of satellite presence on the smallest element of this pair could be obtained (3c).

Monochamus sartor
A female chromosome number of $2n = 24$ has been consistently found (Fig. 4a). The karyotype presents five pairs of metacentrics (1, 3, 4, 5 and 7), two pairs of submetacentrics (6 and 9), four pairs of subacro-acrocentrics (2, 8, 10 and 11) plus an heteromorphic pair (12). The latter according to male meiotic observations was identified as the heterochromosomal pair. Spermatocyte metaphases I have 11 regular autosomal bivalents plus the sex chromosomes associated in the 'parachute' configuration (male meioformula 11 + Xy_p) (Fig. 4b). No spermatogonial mitotic plates were found.

Discussion

The purpose of this study was to provide an initial step in understanding the evolutionary history of the European *Monochamus* taxa on the basis of mitochondrial DNA sequences and karyotype features. With the only exception of karyological data on American taxa, no comparable information exists for this widespread and economically important group of beetles.

As far as molecular analyses are concerned, it should be first noted the lower phylogenetic signal of the 12S gene in comparison with the COI gene; this is obviously due to the lower variability of the small subunit RNA gene sequences and confirms the utility of the COI gene region in Coleoptera at the generic level and below (Funk et al. 1995).

Nevertheless, both genes agree in indicating identity between the two subspecies of *M. galloprovincialis* irrespective of sample geographical origin: for each gene only one haplotype has been scored for the two samples (Table 2). The identity of the two taxa is further supported by chromosome analyses (same karyotype structure). The morphological subdivision of *M. galloprovincialis* in two subspecies is therefore not convincing, and further investigations should be performed to solve the incongruency between gross morphology and molecular/karyological data.

Both genes also agree in supporting a strong affinity between *M. sartor* and *M. urussovi*: their divergence is extremely low when compared with the other haplotypes scored. Taking into account that the two taxa are considered vicariant and have been independently considered synonymous with *M. rosenmuelleri* (Danilevsky 2003), it could be suggested that their differentiation may not be considered of specific level. On the

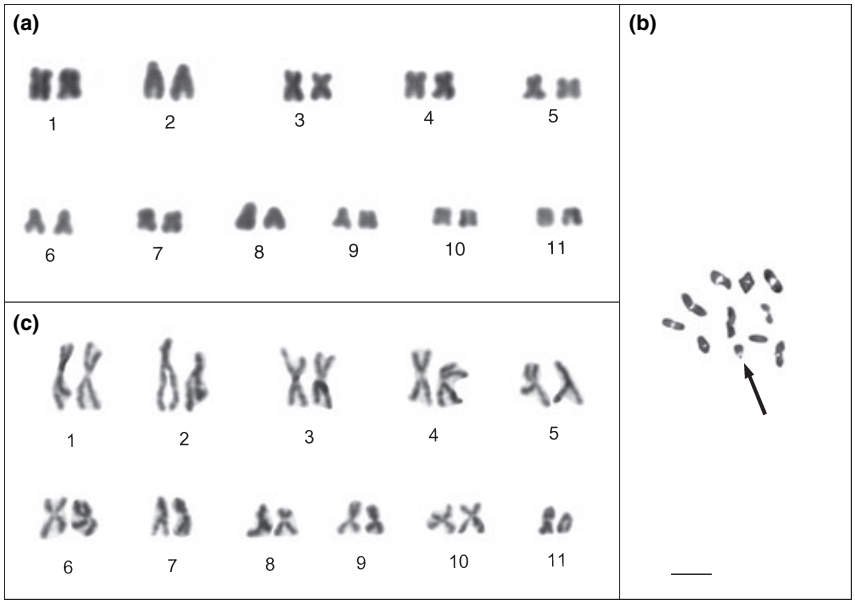

Fig. 3. *Monochamus galloprovincialis*: (a) female Giemsa karyotype of *M. galloprovincialis galloprovincialis*; (b) spermatocyte metaphase I of *M. galloprovincialis galloprovincialis* showing 10 autosomal bivalents plus the Xy_p association (arrow); (c) *M. galloprovincialis pistor* Giemsa karyotype of a somatic cell. Bar represents 5 μm

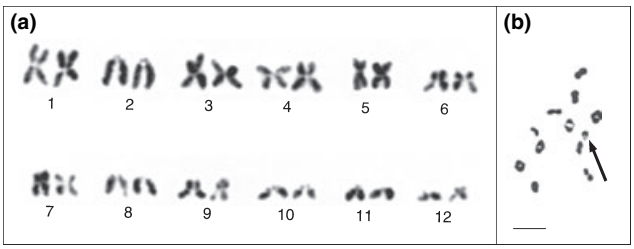

Fig. 4. *Monochamus sartor*: (a) female karyotype Giemsa stained; (b) spermatocyte I nucleus with 11 autosomal bivalents and the Xy_p sex-chromosomes association (arrow). Bar represents 5 μm

other hand, the Italian and the Japanese samples of *M. saltuarius* always cluster together in all scored trees: haplotypes are only slightly differentiated and appear to belong to the same species.

For the remaining taxa, the scored level of differentiation is comparable with that observed in other intrageneric analyses in Coleoptera (e.g. Clark et al. 2001).

Chromosome analyses show interspecific differences as to the number of autosomes (ranging from 18 in *M. saltuarius* to 22 in *M. sartor*) and a fixed heterochromosome formula (Xy_p); this condition contrasts to the one observed in North American *Monochamus* species, where a stability of the autosomal number ($2n = 18$) and a great variation in number and size of sex chromosomes were demonstrated (Smith 1950, 1953; Lanier and Raske 1970).

A chromosomal condition with nine pairs of autosomes, likely euchromatic metacentrics, is the most frequent in Polyphaga and has been reported in some Adephagans; it has been therefore suggested as the primitive condition of the order Coleoptera (Smith 1953; Virkki 1984; Petitpierre 1996). Chromosomal evolution is further characterized by centric fissions, often followed by pericentric inversions or heterochromatin additions, these kind of rearrangements being widespread (Smith and Virkki 1978; Virkki 1984). In contrast, centric fusions appear less frequent, although the karyotypic lowest numbers scored in some Coleoptera seem to have arisen through these structural rearrangements (Virkki 1984).

Among presently analysed taxa, only *M. saltuarius* shows nine pairs of autosomes and agrees with data on American *Monochamus* taxa (Smith 1950, 1953; Lanier and Raske 1970). The *M. galloprovincialis* karyotype could be derived from a *saltuarius*-like chromosome complement through the Robertsonian fission of the biggest element and other small rearrangements. This is suggested by the increase of heterobrachial elements and the decrease of dibrachial chromosomes. The occurrence of two centric fissions in the complement *saltuarius*-like of a common ancestor may have increased the autosomal number to 11 pairs giving origin to a karyotype as that of *M. sartor*.

Although preliminary, karyological observations appear in line with molecular data at the taxonomic level and in indicating a more primitive condition of Euroasiatic *M. saltuarius* with respect to *M. galloprovincialis* and *M. sartor*, which represent two clearly diverging evolutionary units.

Acknowledgements

Authors are grateful for *Monochamus* specimen collections to Dr Klaus Hellrigl, Bressanone (I) (*M. saltuarius*); Dr F. Pennacchio, Dr Marco Vittorio Covassi Istituto Sperimentale per la Zoologia Agraria, Firenze (I) (*M. sutor*, *M. galloprovincialis* and *M. sartor*); Dr Jerzy Gutowski, Forest Research Institute Departments of Natural Forests, Bialowieza (Poland) (*M. urussovi*); Dr Nobuo Ogura, Division of Forest Biology, Forestry and Forest Products Research Institute, Tsukuba (Japan) (*M. alternatus*) and to Dr Jikumaru Shota, Hiroshima Prefectural Forest Research Center Miyoshi (Japan) (*M. saltuarius*). Authors thank Dr Iwona Skrzecz, Forest Research Institute, Bitwy Arsszawskiej of Varsavia (Poland) and Dr Annamaria Leccese, Istituto Sperimentale per la Zoologia Agraria, Firenze (I) for their collaboration in the course of the study. We want to thank also the technician Paola Nisticò for keeping ABI PRISM 310 alive and well.

Zusammenfassung

Taxonomie und Phylogenie der europäischen Monomachus-Arten: Erste molekulare und karyologische Daten

Die weltweit verbreitete Gattung Monomachus Megerle, 1821 (Coleoptera, Cerambycidae) enthält Käferarten, die in den Nadelwäldern der nearktischen und paläarktischen Regionen zu wirtschaftlich bedeutenden Schädlingen werden können. Neben dem direkten Schaden durch die minierenden Larven sind sie auch als Hauptvektoren des Nematoden *Bursaphelenchus xylophilus* bekannt geworden. Wir haben das gesamte Gen der mitochondrialen Cytochromoxidase I und ein Stück des ssRNA-Gens (1536 bp) der fünf europäischen Arten (*M. galloprovincialis*, mit den zwei Unterarten *M. g. galloprovincials* und *M. g. pistor*, *M. sutor*, *M. saltuarius*, *M. sartor* und *M. urossovi*, sowie für einen Vergleich auch von der asiatischen Art *M. alternatus* und aus dem japanischen Verbreitungsgebietes der Art *M. saltuarius* analysiert. Beide Gene erweisen sich für die beiden Unterarten *M. g. galloprovincials* und *M. g. pistor* als vollkommen identisch und weisen auf eine nahe Verwandtschaft zwischen *M. sartor* und *M. urossovi* hin; die morphologische Unterteilung in die beiden Unterarten und die Auftrennung dieser beiden Spezies auf Artniveau wird also genetisch nicht unterstützt. Auf der anderen Seite clustern die italienischen und die japanischen Stichproben von *M. saltuarius* in allen Dendrogrammen sehr eng, so daß ihr Status als Art gegenüber den anderen Taxons nicht bezweifelt werden kann. Vom phylogenetischen Standpunkt beurteilt weist die Topologie der Bäume darauf hin, daß die japanische Art *M. alternatus* die am meisten abweichende Spezies und die eurasische Art *M. saltuarius* die ursprünglichste unter den streng europäischen Monomachus-Arten ist. Die Chromosomenanalyse ergibt, daß die diploide Chromosomenzahl sich von 18 bei *M. saltuarius* auf 20 bei *M. galloprovincialis* und auf 22 bei *M. sartor* erhöht, das geschlechtsdeterminierende System aber bei allen Taxa gemeinsam dem Typ XX-Xy_p entspricht. Der Karyotyp von *M. saltuarius* scheint der ursprünglichste zu sein, von dem die anderen durch Robertsonsche Fissionen abgeleitet werden können. Die karyologischen Daten stimmen auch mit den molekularen überein, indem sie der eurasischen Art *M. saltuarius* eine, zu den europäischen Arten basale Position zuweisen. Unter diesen stellen die Arten *M. galloprovincialis* und *M. sartor* zwei deutlich divergierende evolutionäre Einheiten dar. Außerdem bestätigen die karyotypischen Analysen die molekulare Schlußfolgerung, daß die zwei Unterarten *M. g. galloprovincials* und *M. g. pistor* identisch sind.

References

Brown, J. M.; Pellmyr, O.; Thompson, J. M.; Harrison, R. G., 1994: Phylogeny of *Greya* (Lepidoptera: Prodoxidae), based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. Mol. Biol. Evol. **11,** 128–141.

Clark, T. L.; Meinke, L. J.; Foster, J. E., 2001: Molecular phylogeny of *Diabrotica* beetles (Coleoptera: Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. Ins. Mol. Biol. **10,** 303–314.

Danilevsky, M. L. 2003: Systematic list of longicorn beetles (Coleoptera, Cerambycoidea) of Europe (version march 2003). In: Hoskovec, M.; Rejzek, M. (ed.), Longhorn beetles (Cerambycidae) of the

West Palearctic Region. URL: http://www.uochb.cas.cz/~natur/cerambyx/index.htm.

De Bruijn, M. H. L., 1983: *Drosophila melanogaster* mitochondrial DNA, a novel gene organization and genetic code. Nature **304,** 234–241.

Dominik, J., 1982: Summer control of xylophagous insect pests in Scots pine stands. Sylwan **125,** 111–117.

Dowton, M.; Austin, A. D., 1997: Evidence for AT-transversion bias in wasp (Hymenoptera: Symphyta) mitochondrial genes and its implications for the origin of parasitism. J. Mol. Evol. **44,** 398–405.

Evans, H. F.; McNamara, D. G.; Braash, H.; Chadoeuf, J.; Magnusson, C., 1996: Pest risk Analysis (PRA) for the territories of the European Union (as PRA area) on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. OEPP/EPPO Bull. **26,** 199–249.

Ferreira, A. D.; Cella, D.; Tardivo, J. R.; Virkki, N., 1984: Two pairs of chromosomes: a new low record for Coleoptera. Rev. Brasil. Gen. **7,** 231–239.

Funk, D. J.; Futuyuma, D. J.; Orti, G.; Meyer, A., 1995: Mitochondrial DNA sequences and multiple data sets: a phylogenetic study of phytophagus beetles (Chrysomelidae: Ophraelia). Mol. Biol. Evol. **12,** 627–640.

Goidanich, A., 1972: Monocamo. In: Enciclopedia Agraria Italiana, Ramo Editoriale degli Agricoltori. Roma: xxxx pp. 761–763.

Grodnitskii, D. L.; Morozov, P. P.; Cherkashin, V. P., 1992: Analysis of different populations of the fir cerambycid *Monochamus urussovi* Fisch. Coleoptera, Cerambycidae) based on groups of quantitative characteristics. Entomol. Obozrenie **71,** 800–806.

Hellrigl, K. G., 1971: Die Bionomie der europäischen *Monochamus* – Arten (Coleopt. Cerambycid.) und ihre Bedeutung für die Forst-und Holzwirtschaft. Redia **52,** 367–509.

Howland, D. E.; Hewitt, G. M., 1995: Phylogeny of the Coleoptera based on mitochondrial cytochrome oxidase I sequence data. Ins. Mol. Biol. **4,** 203–215.

Jermin, L. S.; Crozier, R. H., 1994: The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. J. Mol. Evol. **38,** 282–294.

Juan, C.; Petitpierre, E., 1991: Chromosome numbers and sex-determining mechanisms in Tenebrionidae (Coleoptera). In Zunino, M.; Bellés, X.; Blas, M. (eds), Advances in Coleopterology, pp. 167–176. Barcelona: European Association of Coleopterology.

Kumar, S.; Tamura, K.; Jakobsen, I. B.; Nei, M., 2001: MEGA2: Molecular Evolutionary Genetics Analysis Software. Tempe, AZ: Arizona State University.

Lanier, G. N.; Raske, A. G., 1970: Multiple sex chromosomes and configuration polymorphism in the *Monochamus scutellatus-oregonensis* complex (Coleoptera: Cerambycidae). Can. J. Gen. Cytol. **12,** 947–951.

Levan, A.; Fredga, K.; Sandberg, A. A., 1964: Nomenclature for centromeric position on chromosomes. Hereditas **52,** 201–220.

Linit, M. J., 1987: The insect component of pine wilt disease in the United States. In: M. J. Wingfield (ed.), Pathogenicity of the Pine Wood Nematode, pp. 66–73. St Paul, MN: The American Phytopathological Society Press, Symposium Series.

Mardulyn, P.; Whitfield, J. B., 1999: Phylogenetic signal in the COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera; Braconidae): evidence of a high diversification rate in this group of parasitoids. Mol. Phylogen. Evol. **12,** 282–294.

Miura, T.; Maekawa, K.; Kitade, O.; Abe, T.; Matsumoto, T., 1998: Phylogenetic relationships among subfamilies in higher termites (Isoptera: Termitidae) based on mitochondrial COII gene sequences. Ann. Entomolo. Soc. Am. **91,** 515–523.

Morewood, W. D.; Hein, K. E.; Katinic, P. J.; Borden, J. H., 2002: An improved trap for large wood-boring insects, with special reference to *Monochamus scutellatus* (Coleoptera: Cerambycidae). Can. J. Forest Res. **32,** 519–525.

Mota, M. M., 2002: Occurrence of the pinewood nematode *Bursaphelenchus xylophilus* in Portugal and perspectives of the disease spread in Europe. Nematology **4,** 124.

Mota, M. M.; Braasch, H.; Bravo, M. A.; Penas, A. C.; Burgermeister, W.; Metge, K.; Sousa, E., 1999: First report of *Bursaphelenchus xylophilus* in Portugal and in Europe. Nematology **1,** 727–734.

Müller, G., 1949–1953: I Coleotteri della Venezia Giulia. Vol. II: Coleoptera Phytophaga (Cerambycidae, Chrysomelidae, Bruchidae). Trieste: Pubblicazione 4, Centro Sperimentale Agrario e Forestale, Ufficio Agricoltura, Foreste e Pesca G.M.A., 685 pp.

Petitpierre, E., 1996: Molecular cytogenetics and taxonomy of insects, with particular reference to the Coleoptera. Int. J. Ins. Morphol. Embryol. **25,** 115–133.

Posada, D.; Crandall, K.A., 1998: Modeltest: testing the model of DNA substitution. Bioinformatics **14,** 817–818.

Preiss, A.; Hartley, D.A.; Artavanis Tsakonas, S., 1988: Molecular genetics of enhancer of split, a gene required for embryonic neural development in *Drosophila*. EMBO Journal **12,** 3917–3927.

Sama, G., 1988: Coleoptera Cerambycidae. Catalogo topografico e sinonimico. Fauna d'Italia. Bologna, Italy: Edizioni Calderini Bologna, 215 pp.

Sama, G., 2002: Atlas of Cerambycidae of Europe and the Mediterranean Area, Vol. 1. Zlín: Nakladatelství Kabourek, 173 pp.

Serrano, J., 1981: Chromosome numbers and karyotype evolution in Caraboidea. Genetica **55,** 51–60.

Shao, J. W.; Dong, X. W.; Jin, Z. H.; Guan, Y. G., 1988: Evaluation of wood losses caused by longhorns in a log yard. J. North-East Forestry Univ. China **16,** 33–40.

Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Liu, H.; Flook, P., 1994: Evolution weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polimerase Chain reaction primers. Ann. Soc. Ent. Am. **87,** 651–701.

Smith, S. G., 1950: The cyto-taxonomy of Coleoptera. Can. Entomol. **82,** 58–68.

Smith, S. G., 1953: Chromosome numbers of Coleoptera. Heredity **7,** 31–48.

Smith, S. G.; Virkki, N., 1978: Coleoptera. In: John, B. (ed.), Animal Cytogenetics, Insecta. Berlin: Gebruder Borntraeger, pp. 366.

Smith, I. M.; Mcnamara, D. G.; Scott, P. R.; Harris, K. M., 1992: Quarantine Pest for Europe. *C.A.B. International (EPPO)* Europ Med. Plant Protection Org. **1032** pp.

Swofford, D. L., 2001: PAUP*-Phylogenetic Analysis Using Parsimony (*and Other Methods), Ver. 4b. Sunderland, MA: Sinauer Associates.

Vallentgoed, J., 1991: Some Important Woodborers Related to Export Restrictions. Forest Pest Leaflet Number 74. Victoria, Canada: Pacific Forestry Centre.

Virkki, N., 1984: Chromosomes in evolution of Coleoptera. In: Sharma, A. K.; Sharma, A. (eds), Chromosomes in Evolution of Eukaryotic Groups, Vol. II. Boca Raton, FL: CRC Press, pp. 41–76.

Authors' addresses: M. Cesari, O. Marescalchi, B. Mantovani (for correspondence), Dipartimento Biologia Evoluzionistica Sperimentale, Via Selmi 3, 40126, Bologna, Italy. E-mail: barman@alma.unibo.it. V. Francardi, Istituto Sperimentale per la Zoologia Agraria, Sezione di Entomologia forestale, Via di Lanciola 12/A, 50125, Firenze, Italy.