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Phylogenetic relationships between genera *Dorcadion*, *Lamia*, *Morimus*, *Herophila* and some other Lamiinae (Coleoptera: Cerambycidae) based on chromosome and CO1 gene sequence comparison

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Research Paper

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Abstract

A dual molecular and cytogenetic study was performed with the aim to improve the controversial systematic classification of some species of Lamiinae (Coleoptera: Cerambycidae). The karyotypes of species belonging to genera *Morimus, Herophila, Dorcadion, Neodorcadion* and *Lamia* share a number of characters, which differentiate them from other species, belonging to genera *Phytoecia, Parmena* and *Monochamus*. The karyotypes of the last three species comprise 20 chromosomes, mostly metacentric or sub-metacentric, as in the presumed Cerambycidae ancestors. The karyotypes of the former species share many characters derived from the Lamiinae ancestors by a number of chromosome fissions and inversions indicating their monophyly. Comparisons of the CO1 gene sequence also show the monophyly of *Morimus, Lamia, Herophila* and *Dorcadion* and their distant relationship with others. These convergent results allow us to propose a phylogenetic classification of these genera, which places the monospecific genus *Lamia* close to *Dorcadion*, clearly separates *Dorcadion* and *Neodorcadion* and places *Herophila* closer to *Morimus* than to *Dorcadion/Lamia*. The genus *Morimus* is the most derived. CO1 mutations loosely separate the forms *M. asper* and *M. funereus*, which have similar karyotypes and behaviour and copulate in captivity. The form *M. ganglebaueri* may have a *funereus* X *asper* hybrid origin.

Introduction

Lamiinae constitute the largest sub-family of Cerambycidae, one of the largest families of Coleoptera which form the largest order of the animal kingdom. Thus, with more than 21,000 species, Lamiinae represent one of the largest taxonomic animal entities. Latreille created this sub-family in 1825 and considered *Lamia textor* Linnaeus, 1758 for its type species. The genus *Lamia* remained mono-specific with *L. textor* for its single species and was included, in the tribe Lamiini Latreille, 1825. Other tribes, such as Dorcadionini Latreille, 1825, Agniini Thomson, 1864, Monochamini Thomson, 1860 and Phrissomini Thomson, 1860 were further created, and shared variously some of the species initially included in the Lamiini. Today, the general trends are either to transfer species belonging to Lamiini to Monochamini or include all Monochamini into Lamiini, but these changes are principally based upon the analysis of phenotypic characters (Bousquet *et al*., 2009; Lobl and Smetana, 2010; Monné *et al*., 2017). Besides *Lamia*, the genus *Morimus* Brullé, 1832 is composed of a small number of Palearctic species/sub-species, which have alternatively been classified into Lamiini or Phrissomini. Phrissomini were isolated from Lamiini to group apterous species but the present trend is to consider this distinction not valid. There is no more unanimity about the distribution of species into these tribes. In Europe, the number of recognized species of *Morimus* varies from 1 to 4, depending on the elevation of three taxons (*ganglbaueri*, Reitter, 1884, *verecundus*, Falderman, 1836 and *funereus*, Mulsant, 1862) to the rank of species, or their classification as simple sub-species of *M. asper* Sulzer, 1776. About ten other species occur in oriental regions. For the sake of simplification, we will arbitrarily call the taxa studied here *M. asper*, *M. ganglbaueri* and *M. funereus*, without presumption of the validity of their species status. The genus *Morimus* shares a number of morphological characters with the genus *Herophila*, Mulsant, 1863. Both are often included in the tribe Phrissomini. Their morphology recalls that of *L. textor* Linnaeus, 1758 and also that of species of *Dorcadion* Dalman, 1817 generally classified into Dorcadionini. Very few cytogenetic studies were

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performed (Smith and Virkki, 1978; Dutrillaux and Dutrillaux, 2011; Okutaner *et al.*, 2011) and there is neither conclusions about the species status within genera, nor the phylogenetic relationships between genera. In a recent study of CO1 and ITS2 genes, developed on *M. asper* and *M. funereus* specimens from Italy, Croatia, Montenegro Slovenia, Turkey and Peloponnese, Solano *et al.* (2013) concluded that their genetic distance is small and compatible with the presence of a single species. The aim of our work was to provide genetic criteria for improving phylogenetic relationships and classification of genera *Morimus, Lamia, Herophila* and *Dorcadion* by the study and comparison of both their chromosomes and their CO1 gene sequence. For genus *Morimus* specimens from two unexplored regions, France and continental Greece were selected. Two of the three species of *Herophila* could also be studied. For genera *Dorcadion*/*Neodorcadion*, the systematic classification is so conflicting that we restricted our report on a few consensual species amongst the many species we studied. Species of three other genera of Lamiinae: *Parmena* (Parmenini), *Monochamus* Dejean, 1821 (Monochamini Gistel, 1848) and *Phytoecia* Mulsant, 1832 (Phytoeciini Mulsant, 1839); are used as outgroups.

Material and Methods

Animals

Morimus asper: male specimens from France, both sides of the Verdon river, near La Palud-sur-Verdon (43°46′48″N, 06° 20′30″E) and near Aiguines (43°46′33″N, 06°14′37″E), and Greece, Tripi, near Sparti (37°04′10″N, 22°16′20′E, fig. 1a) Peloponisos, near Metsovo (39°46′09N, 21°10′55″E, fig. 1f) West Macedonia/Epirus border, Mount Parnassus (38°33′09″N, 22°33′30″E, fig. 1d) Central Greece.

Morimus funereus: male specimens from Greece, Thessaly: Mount Ossa (39°47′59″N, 22°41′13″E, fig. 1h); Mount Olympus (40°05′08″N, 22°21′31″, fig. 1k) and Mount Pelion, near Volos (39°30′N, 22°57′E); West Macedonia: mount Pelister near Kastoria (40°31′N, 21°18′E, fig. 1g); East Macedonia/Thrace bordure: Central Rodopi, Kariofito, near Stavroupoli (40°40′N, 22° 56′E, fig. 1j).

Morimus ganglbaueri Reitter, 1894: One male and one female specimens from Bourazani, near Albanian border (40°03′13″N, 20°37′36″′E, fig. 1l), Epirus, Greece.

Herophila tristis Linnaeus, 1767: male specimens from Greece, near Lake Prespa (40°46′N, 21°06′E), West Macedonia and Bourazani (Fig. 1n, o).

Herophila faimairei Thomson, 1857: female specimens from Mount Parnassus (fig. 1p).

Lamia textor Linnaeus, 1758: one female from the south of France (fig. 1s).

Dorcadion (Cribridorcadion) thessalicum Pic, 1916: males from the Meteora region (39°42′N, 21°37′E, fig. 1t), Thessaly, Greece.

Dorcadion (Cribridorcadion) equestre Laxman, 1770: males from the Ohrid region (41°07′N, 20°48′E, fig. 1u), Republic of Macedonia.

Dorcadion (Cribridorcadion) obenbergeri Heyrovsky, 1940 from Vermio Mountains (40°37′26″N, 21°56′33″E, fig. 1v), Macedonia, Greece.

Dorcadion (Iberodorcadion) fuliginator Linnaeus, 1758: males from the Irun region (43°20′N, 01°47′W, fig. 1m), Spain.

Neodorcadion bilineatum Germar, 1824: males from Mount Pelion, Thessaly (39°26′N, 23°02′ E, fig. 1q) and several other places in Greece.

Neodorcadion exornatum: males from Microderio (41°18′N, 26°01′E, fig. 1r) Thraki, Greece.

Parmena pubescens Dalman, 1817: one female from Methoni (36°50′N, 21°40′E, fig. 1x) Peloponisos, Greece.

Monochamus sutor Linnaeus, 1758: male from Stavroupoli region (41°12′N, 24°42′E, fig. 1y) Thraki, Greece.

Phytoecia (Musaria) affinis Harrer, 1784: male and female specimens from Mount Olympus (40°04′55″N, 22°20′55″ E, fig. 1w).

Cytogenetic techniques

Proliferating cells of three origins were obtained: testicles, mid gut and eggs.

Testicular follicles

Follicles were extracted from the abdominal cavity. Each follicle was immediately dropped into a 0.88 g l^{-1} aqueous KCl solution where it remained for 15 min at room temperature. After centrifugation, it was transferred into a micro-centrifuge tube (VWR International SAS, code 211-0033) containing a 0.55 g l^{-1} KCL solution, where it was squashed and suspended using a piston (VWR, code 045520) adapted to the tube. The volume of the supernatant was increased to 1.5 ml by addition of 0.55 KCl solution. After 10 min, the hypotonic solution was replaced by Carnoy 1 fixative where the cells were suspended and left for a minimum of 30 min. After one change of fixative (all centrifugations 5 min at 800 ***g***), the cells were spread on wet and cold slides. Giemsa staining, C-banding and NOR-staining were performed as described (Angus, 1982, 1988; Howell and Black, 1980; Dutrillaux *et al.*, 2006).

Mid-gut cells

Dissection was performed in 0.88 g l^{-1} KCl. About 0.3 cm long fragments of mid gut were cleared out of their content and put in a watch-glass containing 0.88 KCl solution. The fragments were dilacerated, one drop of colcemid solution was added and the mixture of suspended cells and remaining fragments was left for one hour before being squashed and treated as above, except that the KCl solution was replaced by foetal calf serum diluted in distilled water (1 vol : 2 vol).

Eggs

The main difficulty is the choice of the best moment to obtain dividing cells. We selected the period when the eggs are swelling. Eggs were cleaned up and put directly in a micro-centrifuge tube containing either a 0.55 KCL or diluted serum solution. Then, they were squashed and treated as described above.

All karyotypes and chromosome measurements were performed using the IKAROS device (METASYSTEMS, Germany).

Sequencing analysis of the mitochondrial COI gene

Mitochondrial DNA was isolated from 34 individuals. A 525 bp segment at the 3′ end of the *COI* gene was amplified using the primers C1J 1718 (5′-GGAGGATTTGGAGGTTGATTAGTTCC-3′) and C1N 2191 (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) (Simon *et al.*, 1994). A polymerase chain reaction (PCR) (50 µl) contained 200–500 ng DNA, 10 × Taq buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 50 pmol of each primer and 1 U Taq polymerase (Invitrogen, Carlsbad, CA, USA). The cycling conditions consisted of an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C

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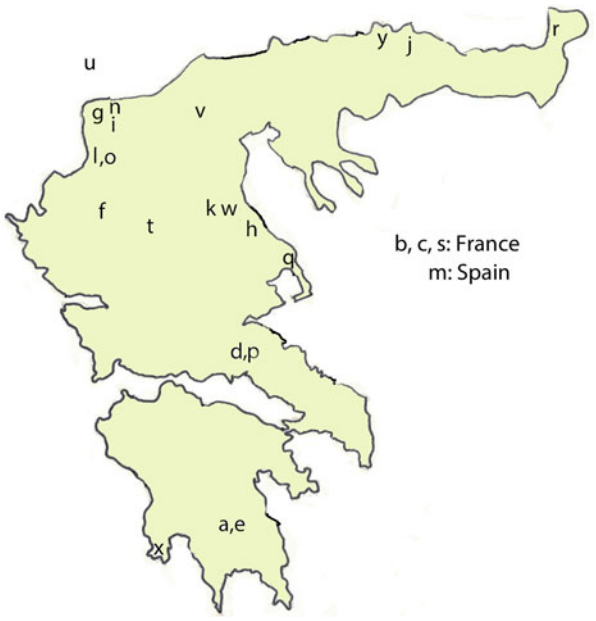

Figure. 1. Map of Greece with indications by letters of the places of sampling. The letters correspond to those indicated in the cladogram of fig. 3: (a–f) *M. asper*; (g–k) *M. funereus*; (l) *M. ganglbaueri*; (m) *D. I. fuliginator*; (n, o) *H. tristis*; (p) *H. fairmairei*; (q) *Neodorcadion bilineatum*; (r) *Neodorcadion exornatum*; (s): *L. textor*; (t) *D. C. thessalicum*; (u) *D. C. equestre*; (v) *D.C. obenbergeri*; (w) *P. affinis*; (x) *P. novaki* and (y): *M. sutor.*

for 40 s and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were purified using a QIAquick PCR purification kit (QIAGEN Cat. No. 28106, Valencia, CA, USA) and were sequenced directly and bi-directionally by Macrogen Inc. Nucleotide sequences were aligned using ClustalX (Larkin *et al.*, 2007).

For all haplotypes, base composition, nucleotide variation, polymorphic and parsimony informative sites, the appropriate model of sequence evolution and phylogenetic associations among lineages were assessed using MEGA version 5 (Tamura *et al.*, 2011). According to BIC scores (Bayesian Information Criterion) the GTR + *G* + *I* model [general time reversible modelled by using a discrete gamma distribution (+ *G*) with five rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+ *I*)] was selected for subsequent analyses and the construction of a neighbour-joining (NJ) tree. Parsimony and Maximum Likelihood (ML) trees were also constructed. Node support was assessed on the basis of 1000 bootstrap replicates. For all trees, the sequence of *Aegosoma scabricorne* was used as the outgroup.

A Bayesian analysis was also performed with MRBAYES version 3.1 (Huelsenbeck & Ronquist, 2001), under the HKY85 model of sequence evolution. Depending on the data set, random starting trees run for 2×10^6 to 8×10^6 generations were used, sampled every 100 generations. Burn-in frequency was set to the first 25% of the sampled trees.

Results

Species identification

All the specimens studied here were captured by in nature. We had no difficulty for determining their specific or sub-specific status, which agreed with the known localities of the species, except for the two specimens of *Morimus* from Bourazani (Epirus, Greece). Their antennae are large and the surface of their elytrons is completely granular, as in *M. asper asper*, whereas the elytra pubescence is pale grey and dark spotted, as in *M. funereus*. These morphologic characters correspond to those described for *Morimus ganglbaueri* Reitter, 1894. However, this species is known from Dalmatian coast (Bosnia, Montenegro and Albania), but not from Greece. Thus, if our identification is correct, this species occurs in Greece, at least in border zones with Albania. It may be also that these specimens are hybrids between *M. asper* and *M. funereus*.

Breeding assays

Morimus imagines, fed with twigs of various species of trees, can be kept alive for several months, i.e., from the month of May until March of the following year in our experience. We kept couples of *M. funereus* and one couple formed by a male *M. asper asper* and a female *M. funereus* captured in nature in Greece. They co-existed peacefully for months with frequent ridings, and their behaviour was similar to that of other couples. Eggs were laid 5 months later, and first instar larvae could colonize a branch of cherry-wood from the Fontainebleau region (where genus *Morimus* does not occur). They were maintained at room temperature and a single female imago was obtained on July. Its phenotype was typically that of *M. funereus*, except that its dark spots were granulated, as in *M. gang*lbaueri and *M. asper asper*, and not *M. funereus*. Unfortunately, we did not know whether or not the female, captured in nature, was virgin. This makes uncertain the hybrid status of the descendant.

Chromosomal data

Morimus asper. Published data: none. The karyotype is composed of 22 autosomes, a sub-metacentric X and a punctiform Y: 24,XY in the males and 24,XX in the females. Three autosomes (1, 2 and 3) are almost metacentric and one autosome (4) is sub-metacentric, with a largely heterochromatic short arm. All other autosomes are acrocentric, with very small amounts of heterochromatin in their short arms (fig. 2.1). This description prevails for specimens of all geographic origins in which only few variations of juxta-centromeric (C-banded) heterochromatin were observed. The most variable segment is the short arm of chromosome 6. At the pachytene stage of meiosis, this arm is elongated, and deeply silver stained indicating it harbours the Nucleolus Organizer Region (NOR). At the first meiotic division, there are 11 autosomal bivalents and the sex bivalent has a parachute configuration: 11 + Xyp.

Morimus ganglbaueri. Published data: none. The karyotype is fairly similar to that described above: 24,XY and 24,XX in the male and female, respectively(fig. 2.2). Heterochromatin is slightly more intensely C-banded and may be present in intercalary position of some chromosomes.

Morimus funereus. Published data: description of a disomy Y: 25,XYY (Dutrillaux and Dutrillaux, 2011). The karyotype, similar in specimens from the different localities, does not look different from that of the previous taxa: 24,XY and 11 + Xyp in the males. Intercalary heterochromatin is occasionally present (chromosome 5, 7) (fig. 2.3).

Herophila tristis. Published data: none. Its karyotype is also composed of 24 chromosomes (24,XY in the males), but

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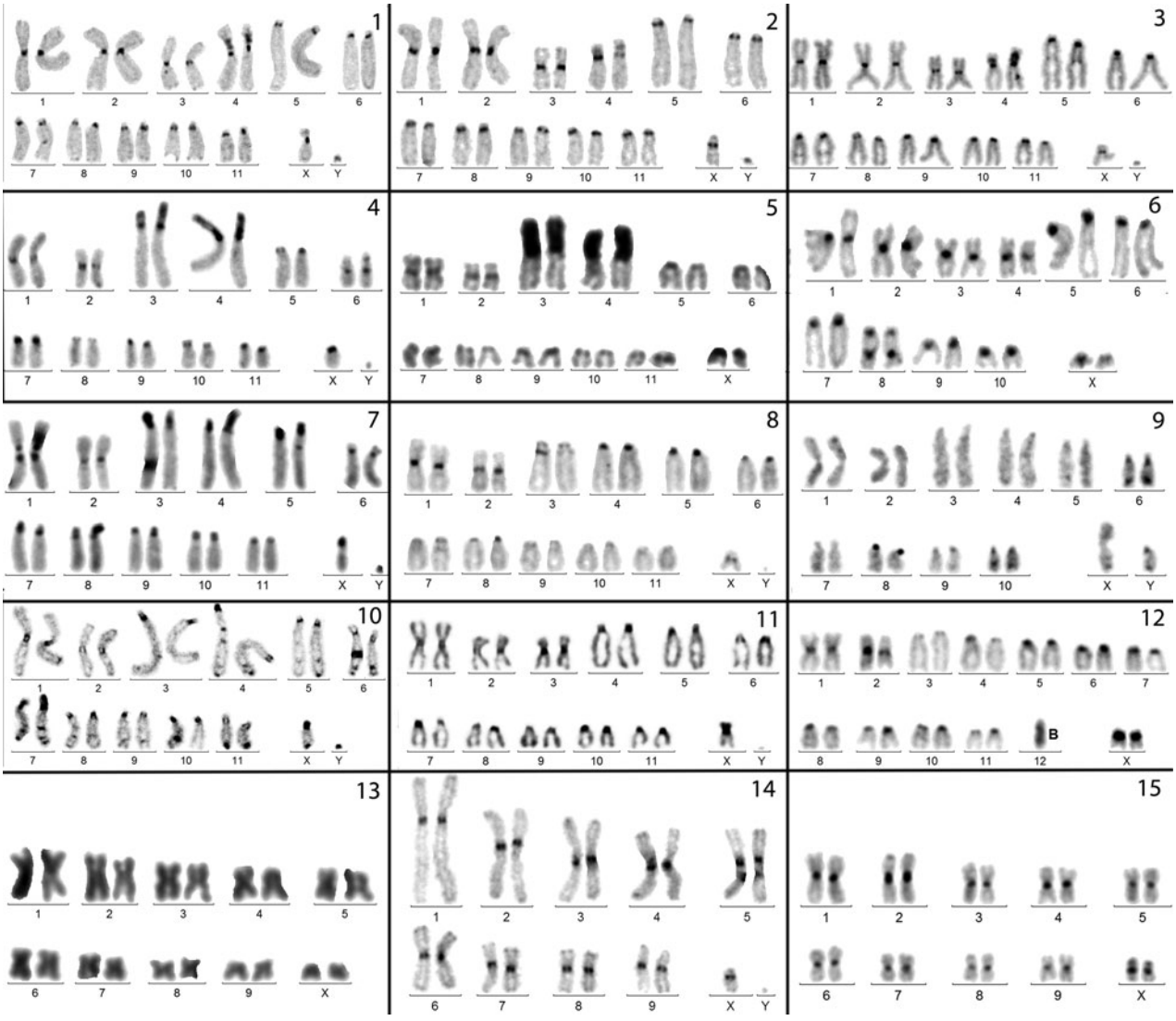

Figure 2. Karyotypes after Giemsa staining (13) or C-banding (all others). (1) *M. asper*; (2) *M. ganglbaueri*; (3) *M. funereus*; (4) *H. tristis*; (5) *H. fairmairei*; (6) *L. textor*; (7) *D. C. thessalicum*; (8) *D. C. equestre*; (9) *D. C. obenbergeri*; (10) *D. I. fuliginator*; (11) *N. bilineatum*; (12) *N. exornatum*; (13) *P. novaki*; (14) *M. sutor* and (15) *P. affinis*.

compared to that of *Morimus* species, only two pairs of autosomes are metacentric (fig. 2.4). The two largest pairs of acrocentrics carry large and variable amounts of heterochromatin, one of them harbouring the NOR. Pair six is acrocentric and harbours heterochromatin in its long arm. Finally, the X chromosome is acrocentric and not sub-metacentric.

Herophila fairmairei. Published data: none. Its karyotype is similar to that of *H. tristis*: 24,XX for the females, but the amounts of heterochromatin on the two pairs of large acrocentrics are yet more important (fig. 2.5). Thus, the amplification of heterochromatin on chromosome 3 and 4 may be a characteristic of the genus *Herophila*.

Lamia textor. Published data: none. Its karyotype is composed of 22 chromosomes only, but one pair (No 8) is either dicentric or pseudodicentric, so that the number of C-banded 'centromeres' is the same as in *Morimus* species (fig. 2.6). Unfortunately, we could not get males, and female karyotypes did not allow us to identify with certainty the X chromosomes. The size and morphology of autosomes recall those of *Morimus*, but the presence of four metacentrics suggests that at least two inversions separate their karyotypes.

Dorcadion (Cribridorcadion) thessalicum. Published data: none. Its karyotype is composed of 22 autosomes, including two metacentrics, an acrocentric X and a punctiform Y: 24,XY in the male (fig. 2.7).

Dorcadion (Cribridorcadion) equestre. Published data: none. Its karyotype is composed of 24 chromosomes, including two pairs of metacentrics, a sub-metacentric X and a punctiform Y: 24, XY in the male (fig. 2.8). The NOR is located on the subcentromeric position of an acrocentric, probably No 6 or 7.

Dorcadion (Cribridorcadion) obenbergeri. Published data: none. Its karyotype is composed of 20 autosomes, a metacentric X and an acrocentric Y (fig. 2.9). The reduction of the number of autosomes and the enlargement of the sex chromosomes are the consequence of a translocation (fusion) between the X proper and an autosome. Thus, the X is a neo-chromosome. Its short arm is formed by the ancestral X, and its long arm is formed by an ancestral acrocentric, whose size corresponds to No 6 or

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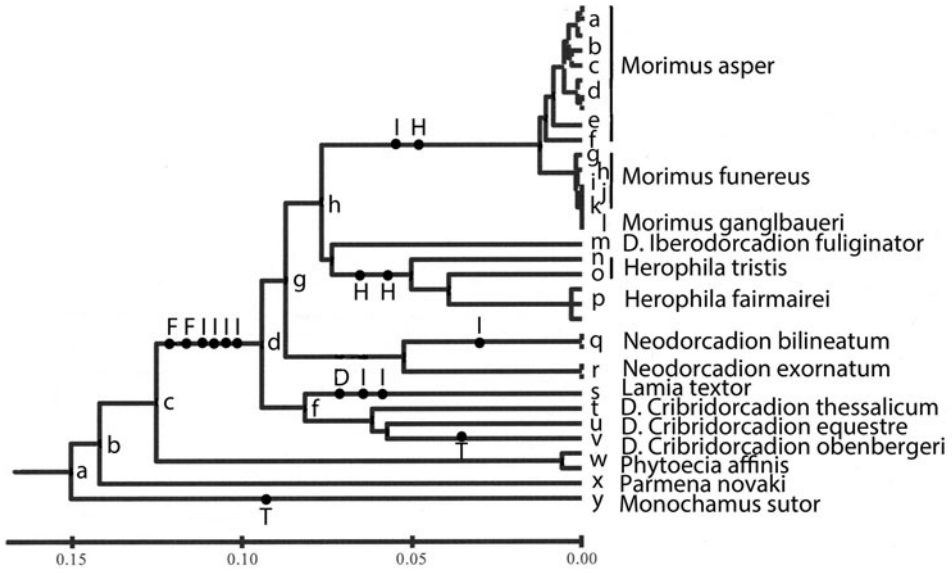

Figure 3. Phylogenetic tree constructed with the NJ algorithm. Small letters on the left correspond to specimens collected at the locations indicated at fig. 1. Chromosome rearrangements separating the karyotypes are indicated but were not considered to establish the tree. F, fission; I, pericentric inversion, T, translocation; D, dicentric or pseudodicentric; H, large heterochromatin addition.

7 in other species of *Dorcadion*. The neoY is probably entirely composed of this autosome. This translocation is a synapomorphy.

Dorcadion (Iberodorcadion) fuliginator. Published data: none. The number and morphology of its chromosomes are quite similar to that *D. equestre*, except for the X, which is acrocentric: 24,XY. After C-banding, most chromosomes exhibit, in addition to the centromeric bands, one or two bands located in the intercalary or terminal position of the chromatids (fig. 2.10). These variable additions of heterochromatin were found in the three males studied.

Neodorcadion bilineatum. Published data: none. Its karyotype is composed of 22 autosomes, with two pairs of metacentrics and one pair of sub-metacentric. Chromosome X is sub-metacentric and the Y is punctiform: 24,XY in the males (fig. 2.11).

Neodorcadion exornatum. Published data: none. Its karyotype is also composed of 22 autosomes with two pairs of metacentrics. The X is sub-metacentric and the Y has a heterochromatic short arm: 24,XY. Thus, the karyotypes of the two species of *Neodorcadion* differ by at least one peri-centric inversion. In the female studied, there was an additional B chromosome (fig. 2.12).

Parmena novaki. Published data: none. Its karyotype is composed of 20 chromosomes, all meta- or submetacentric of progressively decreasing size: 20,XX in the single female studied (fig. 2.13). The C-banding is limited to the centromeric region.

Monochamus sutor. Published data: Teppner (1966), Abe *et al.* (1971), Kudoh *et al.* (1972), Cesari *et al.* (2005) and Dutrillaux and Dutrillaux (2014). The karyotype is composed of 18 autosomes, all meta- or sub-metacentric. The X chromosome is sub-metacentric and the Y is punctiform: 20,XY in the males (fig. 2.14). The NOR is sub-centromeric on chromosome 7. There is a size hiatus between chromosomes 1 and 2.

Phytoecia affinis. Published data: none. Its karyotype is composed of 20 chromosomes, all the autosomes are either metacentric or sub-metacentric of progressively decreasing size: 20,XY in the males and 20,XX in the females (fig. 2.15). The short arm of the X is largely heterochromatic.

Nucleotide analysis and phylogenetic reconstruction

Sequences obtained, primers excluded, corresponded to a 525 bp segment. The absence of insertions, deletions or in-frame stop codons within the sequences studied indicated that they correspond to functional mitochondrial *COI* gene fragments and are not derived from nuclear mitochondrial pseudogenes (numts), commonly occur in most eukaryotic species (Richly & Leister, 2004). Haplotypes were evaluated and compared to. All haplotypes, after evaluation and comparison to published sequences in Genbank, corresponded to new sequences and thus were submitted to GenBank with accession numbers: MH613717-MH613750.

The high percentage (64.7% on average) observed for the A + T content is a common feature of animal mitochondrial genes (Brown, 1985). Out of the 525 sites, 196 were variable while 168 of them were informative for parsimony. Net genetic distances between species ranged from 1.8% (*M. asper–M. funereus*) to 26.3% (*Monochamus sutor–Dorcadion bilineatum*). Nucleotide divergence within *M. asper* and *M. funereus* were 1.2 and 0.2%, respectively. With some minor differences, the phylogenetic trees based on the Bayesian analysis (tree not shown, available on request), as well as on pairwise haplotype divergence (NJ) (fig. 3), showed similar topologies. The main branches exhibited high bootstrap values (NJ) and high posterior probabilities (Bayesian analysis).

Discussion

Chromosome comparisons and evolution

In Cerambycidae, as in Polyphagan Coleoptera in general, the most frequently reported karyotype formulae are 20,XY and 20, XX, in males and females, respectively, with all or almost all chromosomes metacentric or sub-metacentric (Smith and Virkki, 1978; Dutrillaux and Dutrillaux, 2009). Such karyotypes were observed in most of the tribes of Lamiinae we studied. In our yet unpublished data base, we found it in at least one species of the following tribes: Acanthocini, Acanthoderini, Agapanthini, Desmiphorini, Mesosini, Monochamini, Onciderini, Phytoeciini, Pteropliini and Saperdini. Thus, it is assumed to represent the ancestral condition, but some variations exist. They consist in an increase of chromosome number to 22, rarely more, generally accompanied by the presence of acrocentrics, which suggests that fissions of the metacentrics occasionally occurred during

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evolution. In the group of 15 species/sub-species studied here, *P. novaki* and *P. affinis* have conserved a karyotype with 20 chromosomes of progressively decreasing size, probably close to that of the ancestor. With its very large chromosome 1, the karyotype of *M. sutor* is probably derived by a translocation, but it conserved the number of 20 chromosomes. All but 2 of the 12 other species studied here have 24 chromosomes. The ten karyotypes with 24 chromosomes, which look not much different from each other, may derive from the primitive situation by the fission of two metacentrics, which formed four acrocentrics. However, their high number of acrocentrics (7 to 9) implies that three to five peri-centric inversions also occurred. The karyotype with 22 chromosomes of *D. obenbergeri*, obviously apomorphic, derives from that of other *Dorcadion* by a gonosome–autosome translocation. On the whole, the chromosome rearrangements, which originated the resembling karyotypes of genera *Morimus*, *Herophila* and *Dorcadion*, very probably occurred in common ancestors. Thus, these genera form a monophyletic group. The chromosomal relationships between *L. textor* and others are less obvious. The karyotype of this species, with 22 chromosomes, looks intermediary between those of the groups with 20 and 24 chromosomes, but one pair looks dicentric, or pseudo-dicentric, as defined in ISCN (1985), with two primary constrictions and two C-bands (fig. 2.6). Thus, it has 24 potential centromeres, and may derive by a termino-terminal fusion of two acrocentric chromosomes from a karyotype with 24 chromosomes. Because of the frequent involvement of the X chromosome in translocations in Coleoptera (Dutrillaux and Dutrillaux, 2009), as here in *D. obenbergeri*, it is possible that the proper X forms one arm of this pseudo-dicentric.

In conclusion, the lack of available probes makes impossible to perform a precise chromosome identification by *in situ* hybridization, and thus, to reconstruct a phylogeny by chromosome comparison. Nevertheless, the great resemblance of their karyotypes is strong arguments in favour of a monophyletic origin of genera *Morimus*, *Herophila*, *Dorcadion* and *Lamia*, which share similar derived features compared to other Lamiinae. At difference, the karyotypes composed of 20 chromosomes, here observed in *P. novaki, M. sutor* and *P. affinis* share many features, with many other Cerambycids. We conclude that they underwent few gross chromosome changes during evolution.

CO1 sequence comparisons

In a recent study Solano *et al.* (2013) analysed 65 specimens of *M. funereus* sampled in Italy, Balkans and Turkey by applying COI and ITS2 molecular markers. Unfortunately, although they found 39 haplotypes for COI, the overlap between this set of sequences and ours was practically non-existent (of approximately 10 nucleotides), making it impossible to compare between them. However, a striking difference between the two studies was the ten-fold higher nucleotide diversity assessed for *M. funereus* ($\pi = 0.0226$) in comparison to our data ($\pi = 0.002$). A possible explanation for this discrepancy could be the much larger range of sampling in the study of Solano *et al.* (2013).

Sequencing does not contradict chromosome data, but it provides a better resolution. Species belonging to the various tribes of Lamiinae are well separated, and there is a common trunk for genera *Morimus, Lamia, Herophila* and *Dorcadion*. The splitting of *Dorcadion* in *Iberodorcadion, Neodorcadion* and other *Dorcadion* will be discussed elsewhere, with the study of many more species. The two species of *Herophila* are monophyletic, and also well individualized. CO1 sequences of specimens of *H. fairmairei*, a rare and very localized species, appear to be fairly close to each other, while those of *H. tristis*, a largely spread species, are more diverse. Thus, both bio-geographic and genetic data suggest that *H. fairmairei* may be a localized vicariant of *H. tristis*.

The genus *Morimus* is fairly homogeneous, although separated into two groups, with *M. asper* on the one side and *M. funereus* and *M. ganglbaueri* on the other side. CO1 sequences did not accumulate many mutations separating French and Greek specimens of *M. asper*. The position of the French specimens of *M. asper* in the cladogram is among the most derived one. This is compatible with a radiation of the genus *Morimus* from Balkans, or more oriental countries, to western countries such as France and Spain, which would be the extremity of the radiation and the most recently colonized countries. The similarity of haplotypes found in *M. ganglbaueri* and *M. funereus* is interesting. *M. ganglbaueri* shares rather more morphological characters with *M. asper* (long antennas and granulation of elytra), than with *M. funereus* (density and colour of pilosity). By these mixed characters and its geographic repartition, surrounded by that of *M. funereus* on the East and *M. asper*, on the South and West, *M. ganglbaueri* might have a hybrid origin. In this hypothesis, the similarity of mitochondrial CO1 sequence with *M. funereus* could indicate that this species was originally the maternal contributor. Our breeding experiments show that hetero-specific couples can occur, but we could not formally demonstrate their fertility.

As suggested by chromosome data, the CO1 sequence places *L. textor* within the group *Morimus, Herophila* and *Dorcadion*, far from other taxa. More precisely, *L. textor* looks very close to *Dorcadion*.

Comparison between CO1 and chromosomal data

There is no major discrepancy between the two sets of data, but the mutations of the CO1 sequence are much more discriminating than the alterations of chromosomes for establishing a phylogeny. Thus, it was possible use the cladogram obtained with CO1 analysis for indicating the minimum number of chromosome rearrangements necessary to pass from one karyotype to another (fig. 3). The maximum number of chromosome changes should have occurred in the common trunk for *Morimus, Herophila*, *Lamia* and *Dorcadion*, which fits with CO1 data. It persists however two major questionings: (1) *D. I. fugilinator* has the same karyotype than the other species of *Dorcadion*, if we omit heterochromatin variations, although CO1 data place it near *Herophila*. The explanation may be that the karyotype of *Dorcadion* is also that of the ancestors of both *Herophilus* and *Morimus,* but the study of more species of *Iberodorcation* is necessary for more supported conclusions. (2) CO1 data place *L. textor* near Dorcadion whereas its karyotype seems to have conserved more ancestral features. The two inversions indicated after bifurcation (fig. 3) could be reverse, but chromosome interpretation would be simpler if *L. textor* was branched between bifurcations c and d before the acquisition of two of the four inversions. The study of more specimens, males in particular, is urgently needed. By evaluating the convergence of chromosomal and molecular data, we believe that, despite its limitations when applied solely (e.g. selective sweeps, inability to identify hybridization), the CO1 marker provides the required signal to reconstruct the phylogenetic relationships between the genera under study. In the last decade, there is growing evidence about the importance of the CO1 gene and its unique barcoding attributes are believed to have arisen from its implication to the process of speciation (Gershoni *et al.*, 2009).

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The species-specific mitonuclear coadaptations that occur to maintain the functionality of the Oxidative Phosphorylation are thought to be a major force of speciation (Lane, 2009). The CO1 gene possesses a 'special place' in the above mentioned since it encodes for a catalytic subunit of cytochrome c oxidase, which is a key OXPHOS regulator (Hill, 2016). As he proposes: '*…it is no coincidence that the rate-controlling gene in the ECT that can enable respiratory adaptation to novel environments is also an effective DNA barcode for animals*'.

Conclusion

The comparisons of CO1 mitochondrial gene sequences and karyotypes give convergent data, which indicate an undisputable monophyletic origin of genera *Morimus*, *Herophila*, *Lamia* and *Dorcadion*, at a large phylogenetic distance from other genera such as *Monochamus*. This does not support classifications, which put together *Lamia* and *Monochamus* in a same tribe, whatever its name Monochamini, Lamiini or Agniini. The nomenclature problem seems to be historical: *Lamia*, considered as the genus type of Lamiinae, logically should remain in the *Lamiini* tribe. It should better have been grouped with *Dorcadion* (Dorcadionini) and *Morimus/Herophila* (Phryssomini), but instead, it has been always grouped with many other taxa, such as *Monochamus*, which have very loose phylogenetic relationships. Thus, two options can be proposed: either *Lamia* with only a few closely related genera remain in a restricted *Lamiini* tribe, or the tribes *Phryssomini* and *Dorcadionini* are suppressed and their species join these few *Lamiini* to form a monophyletic new *Lamiini* tribe. This would provide a logical basis for further classifications

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