The Chromosomes of Lepturinae (Coleoptera: Cerambycidae) II. A study of 8 more species, with focus on *Desmocerus palliatus*

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Summary. Following the study of 28 species of Lepturinae (Coleoptera: Cerambycidae) the karyotypes of 7 additional Palaearctic and one Nearctic species are established. The 19,X male karyotypes found in genera *Stictoleptura* (4 species)*, Vadonia* and *Judolia* (1 species each) confirm the loss of Y chromosome in Lepturini. The 22,XY male karyotype of *Cortodera humeralis* is very close to that of some species of Rhagiini (genera *Gaurotes*, *Acmaeops, Dinoptera*, all 22,XY) and *Grammoptera ruficornis* (24,XY) recently reported. We propose that these taxa form a monophyletic group within Rhagiini. The karyotype of the Nearctic species *Desmocerus palliatus* (23,neoXneoXneoY) is quite different and characterized by the presence of many acrocentric chromosomes and and a complex autosome-gonosome translocation. Its particular karyotype is compatible with its present classification within a separate tribe, the Desmocerini.

Résumé. Après l'analyse des chromosomes de 28 espèces de Lepturinae (Coleoptera: Cerambycidae), les caryotypes mâles de 7 autres espèces paléarctiques et d'une espèce néarctique sont présentés. Les caryotypes 19,X observés dans les genres *Stictoleptura* (4 espèces), *Vadon*ia et *Judoli*a (1 espèce de chaque) confirment la perte du chromosome Y chez les Lepturini. Le caryotype mâle 22,XY de *Cortodera humeralis* le rapproche de celui des genres *Gaurotes, Acmaeops* et *Dinoptera* , tous également à 22,XY et de celui de *Grammoptera ruficornis* 24,XY récemment rapportés. Ces données indiquent qu'ils forment un groupe monophylétique, probablement dérivé d'ancêtres de Rhagiini, à caryotype 20,XY. Enfin, le caryotype de l'espèce néarctique *Desmocerus palliatus* (23,neoXneoXneoY) est très différent et caractérisé par la présence de nombreux chromosomes acrocentriques et d'une translocation complexe autosome-gonosome d'un type rarement observé. Ce caryotype original est compatible avec sa classification actuelle dans une tribu séparée, les Desmocerini.

Keywords: Lepturinae; chromosomes; classification; *Cortodera*; *Desmocerus*

Lepturinae are composed of about 1,000 identified species, largely distributed over the world, principally in the Northern hemisphere. Their systematic nomenclature and classification remain uncertain and moving. A given species may be alternatively classified into a large variety of genera such as *Leptura* Linnaeus, 1758*, Corymbia* Hill & Jonhson, 1995*, Paracorymbia* Miroshnikov, 1998, *Brachyleptura* Casey, 1913*, Aredolpona* Nakane and Ohbayashi, 1957*, Rutpela* Nakane & Ohbayashi, 1957, *Stictoleptura* Casey, 1927*, Anastrangalia* Casey, 1924, *Strangalia* Audinet-Serville, 1835 etc…, depending on the importance given to such or such morphological character. The imbroglio is not less for the classification into tribes. Their number varies from 5 to 8, and only the existence of Lepturini Latreille, 1802, Rhagiini Kirby, 1837, Desmocerini, Blanchard, 1845, Encyclopini, LeComte, 1873 and Xylosteini Reitter, 1913 makes consensus, although there is no complete agreement on the species which composed them. Lepturini and Rhagiini are by far the two most numerous tribes, in which some genera such as *Cortodera* Mulsant, 1864 and *Grammoptera* Audinet-Serville, 1835 have been alternatively classified. Recently, the chromosome study of males from 28 species of Lepturinae revealed a mixture of XY and X0 sex formulae (Dutrillaux & Dutrillaux, 2018). Almost all species from the other sub-families of Cerambycidae have an XY formula (Smith & Virkki, 1978), which means that the Y chromosome was lost in a group of Lepturinae. Such transmissible chromosome change is hardly reversible and must have a strong systematic value. All X0 males studied belonged to Lepturini, while XY males were found in various tribes. It was concluded that the loss of Y chromosome might be a synapomorphy of the tribe Lepturini (Dutrillaux & Dutrillaux, 2018), but one species, *Grammoptera ruficornis* Fabricius, 1781, presently classified in Lepturini (Lobl & Smetana, 2010) made exception with its XY sex chromosomes. Here, we report chromosome data of 8 more species of Lepturinae, including *Desmocerus palliatus* Forster, 1771, which belong to Desmocerini, a tribe never studied cytogenetically. It is shown that *Cortodera humeralis* shares many chromosomal similarities, including its XY sex formula, with a group of species usually classified into Rhagiini and *G. ruficornis,* usually classified into Lepturini. This provides a strong objective argument to challenge the classification of both genera *Grammoptera* and *Cortodera* into Lepturini. On the basis of their chromosome constitution, we propose that they

form a monophyletic group within Rhagiini, with species of genera *Gaurotes*, *Acmaeops* and *Dinoptera*.

Material and Methods

Insects. Two male specimens of *Desmocerus palliatus* (Desmocerini) were captured in Québec province in July 2005. The other insects studied are generally classified into the tribe Lepturini (Lobl & Smetana, 2010, Tavakilian & Chevillote, 2017). They were collected in Greece in May 2018 and in the South of France in June 2018 (table 1). For *Stictoleptura hybrida*, the eggs of a single female were studied. For other species, 3 to 5 male specimens were dissected.

Cytogenetic techniques. Following anaesthesia by ethyl acetate, mid-gut and testicular follicles were dissected and dropped into an aqueous solution of 0.88 M KCl (Potassium Chloride) where they remained for 15 min at room temperature. They were transferred into a micro-centrifuge tube (VWR International SAS, code 211-0033, Strasbourg, France) containing either 0.5 ml of 0.55 M KCl (hypotonic) solution or diluted foetal calf serum (distilled water 3 vol. and serum 1 vol.), where they were squashed and suspended using a piston pellet (VWR, code 045420) adjusted to the internal diameter of the tube. The volume of the hypotonic solution was completed to 1.5 ml. After 10 min, the cell suspension was centrifuged during 5 min at 800 g. The supernatant was replaced by Carnoy I fixative, in which the cells were suspended and left for at least 30 min. After one change of fixative, the cells were spread on wet and cold slides or conserved for a few days before use. Slides were stained by Giemsa (2% in water) and photographed. They were further C-banded according to Angus (2008).

Results

Gametogenesis.

In all adult specimens, but *D. palliatus*, gametogenesis was achieved at the time of capture, and only spermatozoa were found in the tests. In *D. palliatus*, all the stages of gametogenesis were still present, although this species was captured later in season.

Chromosomes.

Genus *Stictoleptura.* The 4 species studied here have quite similar karyotypes, composed of 19 chromosomes in the male, including a single sex chromosome: 19,X. As in other Lepturini (Dutrillaux & Dutrillaux, 2018), pair N° 1 is much larger than the other autosomes, which have a progressively decreasing size (Fig. 1 A-D). All the chromosomes are metacentric or sub-metacentric. As often in Lepturini, it is difficult to obtain a C-banding, which remains faint and often limited to some centromere regions.

Vadonia bisignata. All the chromosomes are metacentric or sub-metacentric, with a size hiatus between chromosome 1 and others. After C-banding, all the centromere regions are stained, but with variable intensities. As for genus *Stictoleptura*, the Y is missing: 19,X (Fig.1 E).

Judolia sexmaculata. Its karyotype has the same characteristics as that of species of *Stictoleptura* and *Vadonia*: 19,X (Fig. 1 F).

Cortodera humeralis. Its karyotype is quite different from the preceding ones. It is composed of 22 chromosomes, including one minute Y chromosome: 22,XY. There is no size hiatus between pair N°1 and others, and 4 pairs of autosomes are acrocentric (Fig 1 G).

Desmocerus palliatus. Compared to that of other species, its karyotype is much more difficult to decipher. It is composed of 23 chromosomes, of which 3 of unequal sizes cannot be paired and probably represent neo sex chromosomes (**a**, **b**, **c**). To understand how they are dispatched in gametes, we made karyotypes with brother spermatocytes II composed of 11 and 12 chromosomes. The largest and the smallest neo sex chromosomes (**a** and **c**) were identified in 18/22 spermatocytes II with 12 chromosomes (Fig. 2). They were considered as the neoXs. The medium sized one (**b**) was identified in 15/17 spermatocytes II with 11 chromosomes. It was considered as the neoY. Pair N° 1 is very large, but its larger arm is principally composed of C-banded heterochromatin (Fig.3). Three other autosome pairs are metacentric and the 6 others are acrocentric, with various amounts of C-banded heterochromatin in the centromere region. Many telomeric regions are also C-banded (Fig. 2, 3) In spermatocytes at metaphase I, there are apparently 11 bivalents and one monovalent. However, after prolonged C-band treatment, as after silver staining, the monovalent is more or less loosely, but systematically, linked to the same bivalent by a structure presumably composed of proteins (Fig. 4 A, B). By analogy with beetles with XY sex chromosomes and a parachute sex bivalent Xy_p, this structure is probably of nucleolar origin. Thus, the

apparent bivalent **a-b** and monovalent **c** form an atypical sex trivalent **a-b-c**, in which: **a** and **b** are linked by one (Fig. 4B) or 2 chiasmata (Fig. 4A) in their long arms composed of autosomal material, and **b** and **c** are linked by nucleolar proteins. Thus, **a** and **c** are the neoXs and **b** is a the neoY, but it remains difficult to accurately reconstruct the rearrangement which occurred.

Discussion

The scarcity of the published data on Lepturinae chromosomes, and particularly on the males (Smith, 1953, Ehara, 1956, Teppner, 1968, Dutrillaux & Dutrillaux, 2018) is caused by the high difficulty for obtaining dividing cells. In most species, male gametogenesis ends before the emergence of the imago (Edwards, 1961, Dutrillaux, 2008). This makes necessary to either work on pre-imaginal stages, often difficult to identify, or analyse somatic cells, which have a very low proliferating rate in the imago. It explains the modest quality of the pictures obtained and the quasi-absence of publication on meiotic stages. In rare exceptions however, the gametogenesis period is delayed and persists in the young imago, as in genera *Acmaeops*, *Gaurotes*, *Grammoptera* and *Desmocerus* (Smith, 1953, Ehara, 1956, Dutrillaux & Dutrillaux, 2018 and this study). Here, we show that the males of 4 species of the genus *Stictoleptura* have fairly similar X0 karyotypes, which confirms our previous findings about Y chromosome loss in Lepturini. Three other species from 3 different genera usually classified in Lepturini were also studied: *J. sexmaculata*, *V. punctata* and C. *humeralis*. The 19,X karyotype of the 2 former species is similar, or very close to that of *Stictoleptura*, whereas that of the last one largely differs by the number and morphology of autosomes and the presence of a Y chromosome. This indicates the proximity of genera *Stictoleptura, Vadonia* and *Judolia* and their larger genetic distance from the genus *Cortodera*. The present results, added to those previously obtained in chromosome studies of Lepturinae, are summarized in Table 2. It shows the clear separation of studied Lepturini from other Lepturinae, in relation with their low chromosome numbers, following the loss of their Y chromosome. At difference, 3 chromosome profiles are found in the species of Rhagiini that we studied: that of the genus *Rhagium*, that of the group *A. pratensis, C. humeralis, D. collaris, G. virginea* and *G. ruficornis* and that of other species.

The position of *Cortodera humeralis*. Interestingly, the karyotype of *C. humeralis* is very similar to that of some species classified in Rhagiini: *Acmaeops pratensis, Gaurotes virginea* and *Dinoptera collaris* (Dutrillaux & Dutrillaux, 2018). In the past, the genus *Cortodera* has been alternatively classified in Lepturini or Rhagiini,

but recent classifications rather favour its position in Lepturini. A molecular phylogeny however, based on CO1 gene sequencing, proposed its classification in Rhagiini, close to *A. pratensis, G. virginea* and *D. collaris* (Sykorova, 2008). These data, fully compatible with the chromosome data, provide independent and strong arguments to bring genus *Cortodera* into Rhagiini rather than Lepturini. More precisely, the four genera *Acmaeops, Cortodera, Dinoptera* and *Gaurotes* constitute a monophyletic group, which was differentiated from other Rhagiini by at least one rearrangement of chromosomes, which increased their number to 2n = 22. We also mentioned that male gametogenesis is delayed until the imago stage in this group, but this could not yet be confirmed for the males of *C. humeralis*.

The position of *Grammoptera ruficornis*. As *Cortodera*, genus *Grammoptera* was alternatively classified in Lepturini or Rhagiini, with a preference for Lepturini in recent classifications (Tronquet, 2014). This is in agreement with the CO1 sequence data, which place *Grammoptera* amongst Lepturini (Sykorova, 2008). Obviously, this does not fit with our chromosome data. This species has 24 chromosomes, including a Y chromosome, which is lost by all (22/22) "other" Lepturini that we studied. The presence or absence of the Y chromosome probably has no incidence on the phenotype and thus on the morphological characters used for classifications. However, it seems to have a great importance for the mode migration of chromosome X at anaphase I of meiosis. The lack of Y prevents the formation of the parachute structure, in which the X and Y are associated through nucleolar proteins, present in all XY males of Coleoptera (Smith and Virkki, 1978). This requires other relationships between the X and nucleolar proteins (Dutrillaux & Dutrillaux, 2017). Furthermore, the loss of Y is not reversible, at difference with gene mutations and chromosome rearrangements. Thus, several hypotheses might explain the presence of a Y chromosome in the karyotype of *G. ruficornis*:

1- The genus *Grammoptera* has been misclassified in Lepturini.
2- Its lineage was isolated very early during Lepturini evolution, before the loss of the Y, which involved all other lepturini studied so far.
3- Its ancestors followed a typical Lepturini evolution, with Y chromosome loss, but this chromosome was later re-introduced by the hybridization of a female (which would fit with data on mitochondrial CO1) with a XY male from another tribe.

This last interpretation is attractive, but the number of chromosomes, above 20, and the size reduction of chromosome 1 bring the karyotype *G.ruficornis* close to that of the group *Acmaeodera, Dinoptera, Gaurotes* and *Cortodera*, thus amongst Rhagiini. Obviously, sequence comparisons of nuclear DNA are needed to propose definitive conclusions.

Presence of a monophyletic group amongst Rhagiini. There is some variability in the karyotypes of Rhagiini, but many species share a 20,XY male karyotype. It is principally composed of non-acrocentric chromosomes, as in most other species of Cerambycidae, and is assumed to be close to the karyotype of polyphagan ancestors (Dutrillaux & Dutrillaux, 2009). Amongst Rhagiini, the group of species from genera *Acmaeops, Cortodera, Dinoptera, Gaurotes and Grammoptera* is clearly individualized by its higher chromosome number (22-24) and the distribution of chromosome sizes. This indicates that at least one chromosome rearrangement, possibly a fission, occurred in a common ancestor, separating them from other Rhagiini. Interestingly, gametogenesis, which ends at the pre-imaginal stage in other Lepturinae (Edwards, 1961, Ehara, 1956), is prolonged in the young imago in species of this group. Considering these characters, we propose that these genera form a monophyletic group derived from Rhagiini ancestors.

The position of *Desmocerus palliatus*. Surprizingly, in cladograms proposed by Sykorova (2008), *D. palliatus* was placed into "Lepturini", near genera *Grammoptera, Etorofus* and *Eustrangalia*. The complex karyotype of this species is very different from that of other Lepturinae, but its interpretation may be misleading. It has an odd number of chromosomes (2n = 23), no minute Y chromosome and a chromosome 1 much larger than the others. These criteria could bring it close to Lepturini, but a more precise analysis discards this possibility. The large size of chromosome 1 is the consequence of C-banded heterochromatin amplification, whereas the large chromosome 1 of Lepturini is composed of euchromatin: thus, their origins are different. The odd number of chromosomes is the consequence of a complex gonosome-autosome translocation, which leads to a chain trivalent at metaphase I of meiosis. Nucleolar proteins are included in this trivalent. Such a link between the X and Y through nucleolus at metaphase I is known only in the parachute structure (Xy_p) present in most species of Coleoptera, including Rhagiini but not Lepturini. Finally, many autosomes are acrocentric (Table 2), whereas all or almost all autosomes are metacentric in Lepturini. Thus, the originality of the karyotype of *D. palliatus* is

compatible with its position in a separate tribe, the Desmocerini, (Bouchard et al., 2011, Tavakilian and Chevillote, 2017), but certainly not in Lepturini. Its number of chromosomes, above 20, and its late gametogenesis may link *D. palliatus* to the group of Rhagiini (*Acmaeops, Gaurotes*, *Dinoptera* and *Grammoptera*) with 22-24 chromosomes, but this may a convergence. We could not precisely reconstruct the rearrangement, which originated its 3 neo-sex chromosomes. In all the species of Coleoptera with a gonosome-autotome translocation, the ancestral Y is probably lost and the 2 neo-sex chromosomes are not linked by nucleolar proteins at metaphase I, but by a chiasma, which occur in their autosomal component (Dutrillaux & Dutrillaux, 2009 and additional personal data). In *D. palliatus*, 2 of the 3 derivative chromosomes are linked by nucleolar material, which suggests that the sites of linkage with the nucleolus of both ancestral chromosomes X and Y are maintained. This system, which is similar or close to that described in a Scolytid and some Chrysomelids (Smith and Virrky, 1978), remains a rare form of autosome-gonosome translocation in beetles.

In conclusion, this study increases to 13 the number of genera and 23 the number of species of Lepturini studied with a 19,X karyotype, which supports a little more the hypothesis that the loss of chromosome Y is a synapomorphy of this tribe. The 22,XY karyotype of *C. humeralis* discards its position in Lepturini, but brings it close to a group of Rhagiini, composed of genera *Acmaeops, Gaurotes* and *Dinoptera*, which share other similar chromosomal characters. In addition, the 24,XY karyotype of *G. ruficornis* is probably derived, but close to this 22,XY karyotype. Thus, we propose that genera *Acmaeops, Gaurotes, Cortodera, Dinoptera, Grammoptera* form a monophylic group, well separated from other Rhagiini by their chromosome constitution. Finally, it is shown that the karyotype of *D. palliatus* is characterized by the presence of a rare form of autosome-gonosome translocation.

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Legendes

Table 1 List of species studied with their current tribe position (L: Lepturini; D: Desmocerini), locations of capture and tissues which provided us with proliferating cells.

Table 2 Summary of data on chromosome number and morphology from 38 species of Lepturinae reported in Dutrillaux & Dutrillaux (2018) and this study. 2n = diploid chromosome number in the male; ND: only female studied (20,XX); Y+ or -: presence or absence of the Y; X: Morphology of the X: SM: Sub-Metacentric, M: Metacentric, A: Acrocentric; 1>>2: size hiatus between chromosomes 1 and 2; 1,2 >3: size hiatus between chromosomes 2 and 3; 1≅2≅3: no size hiatus between chromosomes 1, 2 and 3; NA: number of acrocentric autosomes and in the bracket, N° of the autosome(s) involved. Hetero: presence of large fragments of heterochromatin; neo: neochromosome.

Figure 1 Giemsa stained karyotypes of A) *S. cordigera*, B) *S. fulva*, C) *S. hybrida*, D) *S. pallens*, E) *V. bisignata*, F) *J. sexmaculata*, G) *C. humeralis.*

Figure 2 Spermatocytes II of *D. palliatus.* Top*:* karyotype made with 2 brother spermatocytes. Each chromosome is shown twice, after Giemsa staining (center) and C-banding (right and left). The chromosomes from the 12,neoX**a** neoX**c** spermatocyte are placed on the left and those of the 11,Y**b** spermatocyte are placed on the right. Bottom: the 2 spermatocytes II (A and B) used for establishing the karyotype.

Figure 3 C-banded karyotype of a spermatogonium of *D. palliatus*, exhibiting the 3 neo sex chromosomes (**a**, **b**, **c**). Inset: Pair N° 1 from another cell, submitted to a prolonged C-banding, which demonstrates the heterochromatic (h) constitution (containing repeated DNA) of its long arms.

Figure 4 Diakineses/metaphases I of *D. palliatus* after Giemsa staining (left) and C-banding (right). The apparent monovalent **c** and bivalent **a**-**b** (left) are

in fact linked and form an atypical neosex trivalent **a-b-c** (right). Chromosomes **a** and **b** are linked by a single terminal chiasma in A (top) or 2 chiasmata in B (bottom, less frequent configuration). Bivalent 1 exhibits a single chiasma in its euchromatic portion in A and 2 terminal chiasmata (ring) in B. Bar = 10 μm