# Chromosome analyses challenge the taxonomic position of *Augosoma centaurus* Fabricius, 1775 (Coleoptera: Scarabaeidae: Dynastinae) and the separation of Dynastini and Oryctini

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## ABSTRACT

*Augosoma centaurus* Fabricius, 1775 (Melolonthidae: Dynastinae), one of the largest Scarabaeoid beetles of the Ethiopian Region, is classified in the tribe Dynastini MacLeay, 1819, principally on the basis of morphological characters of the male: large frontal and pronotal horns, and enlargement of fore legs. With the exception of *A. centaurus*, the 62 species of this tribe belong to ten genera grouped in Oriental plus Australasian and Neotropical regions. We performed cytogenetic studies of *A. centaurus* and several Asian and Neotropical species of Dynastini, in addition to species belonging to other sub-families of Melolonthidae Leach, 1819 and various tribes of Dynastinae MacLeay, 1819: Oryctini Mulsant, 1842, Phileurini Burmeister, 1842, Pentodontini Mulsant, 1842 and Cyclocephalini Laporte de Castelnau, 1840. The karyotypes of most species were fairly alike, composed of 20 chromosomes, including 18 meta- or sub-metacentric autosomes, one acrocentric or sub-metacentric X-chromosome, and one punctiform Y-chromosome, as that of their presumed common ancestor. Among the Dynastinae we studied, the karyotypes of the two species of *Oryctes* Illiger, 1798 and *A. centaurus* deeply differ from others: they look alike and are composed of 18 chromosomes only, many acrocentric autosomes and neo-sex chromosomes resulting from an X-Y-autosome translocation. The reassessment of their morphologies, in particular of female specimens, exhibits many shared characters. The genetic proximity of *Augosoma* and *Oryctes* is confirmed by the comparison of DNA sequence data separately found in the literature. Thus, the genera *Augosoma* and *Oryctes* should be grouped together, and not split into two different tribes. These results challenge the separation of the tribes Dynastini and Oryctini and the traditional use male exaggerated characters for establishing a systematic classification.

**KEY WORDS**
similarity,
chromosomes,
DNA,
*Augosoma*,
*Oryctes*,
Dynastinae,
revision.

## RÉSUMÉ

*L'analyse chromosomique remet en cause la position taxonomique d'*Augosoma centaurus *Fabricius, 1775 (Coleoptera : Scarabaeidae : Dynastinae) et la séparation des Oryctini et Dynastini*

*Augosoma centaurus* Fabricius, 1775 (Melolonthidae: Dynastinae), l'un des plus grands coléoptères Scarabaeoidea de la Région Éthiopienne, est classé dans la tribu des Dynastini MacLeay, 1819, principalement sur la base des caractères morphologique du mâle : grandes cornes frontales et pronotales et allongement des pattes antérieures. Les 62 autres espèces de cette tribu sont regroupées en dix genres localisés dans les régions orientale, australiasienne et néotropicale. Nous avons réalisé une étude chromosomique d'*A. centaurus* et de plusieurs autres espèces de Dynastini d'Asie et d'Amérique, ainsi que d'espèces appartenant à d'autres sous-familles de Melolonthidae Leach, 1819 et de plusieurs tribus de Dynastinae MacLeay, 1819 : Oryctini Mulsant, 1842 ; Phileurini Burmeister, 1842 ; Pentodontini Mulsant, 1842 et Cyclocephalini Laporte de Castelnau, 1840. Les caryotypes de la plupart des espèces étudiées sont assez semblables, avec 20 chromosomes, composés de 18 autosomes méta- ou sub-métacentriques, d'un X acrocentrique ou sub-métacentrique et d'un Y ponctiforme, comme celui de leurs ancêtres communs. Parmi les Dynastinae, les caryotypes des deux espèces d'*Oryctes* Illiger, 1798 étudiées et d'*A. centaurus* font exception : ils sont très semblables et comprennent 18 chromosomes, de nombreux autosomes acrocentriques et des neo-chromosomes sexuels résultant d'une translocation X-Y-autosome. La morphologie des mâles d'*A. centaurus* évoque celle des autres Dynanastini, mais celle des femelles est beaucoup plus proche du genre *Oryctes*. Devant cette ambiguité, nous avons regroupé et analysé les données publiées du séquençage de l'ADN, qui confirment pleinement la grande proximité *Oryctes-Augosoma*. Ceci remet en cause la classification actuelle de ces genres dans deux tribus différentes, ainsi que la séparation des Oryctini et des Dynastini et la prise en compte des caractères exagérés des mâles pour établir une classification systématique.

**MOTS CLÉS**
similarité,
chromosomes,
ADN,
*Augosoma*,
*Oryctes*,
Dynastinae,
révision.

# INTRODUCTION

Dynastinae MacLeay, 1819 have been long classified among the family Scarabaeidae Latreille, 1802, but various attempts for changing this classification were published, proposing for instance to elevate the Melolonthinae Leach, 1819 sub-family to the family level (Baraud 1992). Recent molecular data (Smith *et al.* 2006; Hunt *et al.* 2007) have provided new insights in this classification, showing that, within Scarabaeoidea, Dynastinae, Rutelinae MacLeay, 1819, Melolonthinae, Orphninae Erichson, 1847 and Cetoniinae Leach, 1815 form a monophyletic group distinct from Scarabaeinae, Glaresidae Kolbe, 1905, Hybosoridae Erichson, 1847, and Aphodiinae Leach, 1815. This could provide an argument to form two families: one of Melolonthidae Baraud, 1992 for the first group and the other of Scarabaeidae, as proposed by Baraud (1992), but this was not adopted by Smith *et al.* (2006). Nevertheless, we will follow the classification of Baraud (1992) which is more in line with molecular data (Hunt *et al.* 2007). Dynastini MacLeay, 1819 is one of the eight tribes of Dynastinae, characterized by a large size and a strong sexual dimorphism, with large frontal and pronotal horns and enlarged fore legs in the males. These features, either associated or not, are however also found in species from other tribes of Dynastinae such as Oryctini Mulsant, 1842 and Agaocephalini Burmeister, 1847 (Endrödi 1985). The geographical distribution of Dynastini is the following. A first group of 18 species, belonging to six genera, spreads over all tropical and adjacent regions of Australasia, from India to New Caledonia. A second group of 43 species, belonging to three genera, occurs from the south of USA to the north of Argentina, with a concentration of species in the northern part of South-America and Central-America (Endrödi 1985). This double localisation evokes a Gondwanan origin, and in this hypothesis, the presence of Dynastini representatives in Africa, such as *Augosoma centaurus* Fabricius, 1775, which linked Indian and South American territories in Pangaea, is expected. However, *A. centaurus* would have remained the only representative of Dynastini, in a continent very rich in species belonging to other Dynastinae tribes, such as Oryctini (52 species) and Pentodontini Mulsant, 1842 (164 species). Cytogenetic studies performed in the sixties and seventies (review in Smith & Virkki 1978 and Yadav *et al.* 1979) gave information mostly limited to chromosome numbers in 20 Dynastinae species. We reassessed chromosome analyses of some species of Dynastinae, belonging to Dynastini principally, and also representatives of Oryctini, Phileurini Burmeister, 1847 and Pentodontini Mulsant, 1842. Their karyotypes were compared to those of species belonging to other sub-families of Melolonthidae, such as Cetoniinae Leach, 1815, Rutelinae, Melolonthinae and Euchirinae Hope 1840, as well as representatives of other families of Scarabaeoidea. In each taxonomic group, 20,XY/20,XX karyotypes, composed of 18 meta-/sub-metacentric autosomes, an acrocentric X and a punctiform Y were found (Dutrillaux & Dutrillaux 2012 and unpublished). This led to the conclusion that such karyotypes represent those of the common ancestors of Scarabaeoidea. A particularity of some species is the reduction of the chromosome number associated with an increase of the X and Y size, interpreted as the result of X-Y-autosome translocation. The resulting neo sex-chromosomes were found in species belonging to different genera of Dynastinae: *Dynastes* Kirby, 1825*, Oryctes* Illiger, 1798*, Cyphonistes* Burmeister, 1847, *Chalcosoma* Hope, 1837 and *Phileurus* Latreille, 1807. The size and morphology of the neoX and Y vary, indicating the involvement of different autosomes in the translocations, thus that the neo sex chromosomes originated from different events of chromosome rearrangements. In the genus *Dynastes*, strictly South-American species have a 20,XX/XY karyotype, while *D. hercules* Linnaeus, 1758 and Northern species have a 18,neoXY/neoXX karyotype. It was concluded that the neo sex chromosomes were derived characters and that the genus *Dynastes* originated in South-America, where it had a 20,XY karyotype and the acquisition of the neo sex-chromosomes created an impassable gametic barrier between Southern and Northern species (Dutrillaux & Dutrillaux 2013). To explore the possible Gondwanan origin of Dynastini, we studied the chromosomes of some Asian species and of *A. centaurus* from Africa, which might represent a link between Asian and American species. We show that the karyotype of *A. centaurus* differs strongly from that of other Dynastini, but resembles that of *Oryctes* species. In *A. centaurus*, although exaggerated morphological characters of the males indisputably recall those of Dynastini, most features of the females rather recall those of the genus *Oryctes*. Both the use of exaggerated characters of the males for establishing systematic or phylogenetic classifications and the Gondwanan origin of Dynastini are discussed.

## MATERIAL AND METHODS

### ABBREVIATIONS

| | |
|---|---|
| 20,XY | male karyotype formula, with 20 chromosomes, X and Y sex chromosomes included; |
| C-banded | Constitutive heterochromatin staining; |
| coll. B.D. | Bernard Dutrillaux's collection; |
| ML trees | Maximum Likelihood trees; |
| neoXY | X and Y chromosomes derived from ancestral sex chromosomes, usually after translocation of autosomes; |
| NOR | Nucleolus Organizer Region. |

### CHROMOSOME ANALYSES

Eventual published data on chromosomes of the species studied are given in the results section. Proliferating cells obtained from either eggs, testes or mid gut were treated as described (Dutrillaux *et al.* 2010). Their chromosomes were Giemsa and further silver stained for localization of the Nucleolus Organizer Region (NOR) and C-banded for localization of heterochromatin. Image capture and karyotyping were performed using IKAROS software (Metasystems, Germany). Chromosome nomenclature: to avoid ambiguous interpretations, we will call acrocentric all chromosomes with a single euchromatin carrier arm, whatever the size of the heterochromatin (generally C-banded) forming the other arm. Chromosomes with euchromatin (not C-banded) on both arms are meta or submetacentric.

### SEQUENCE ANALYSES

In this work, we did not perform any DNA sequencing, but we simply combined two sets of data published independently (Hunt *et al.* 2007; Rowland & Miller 2012), to confirm the cytogenetic findings. This is why sequence analyses will be presented in the discussion section only. The accession number of all sequences retrieved from GenBank is given in Fig. 4. For all sequences, phylogenetic associations were assessed with PAUP* 4.0 beta 10 version (Swofford, 1998). The program MODELTEST Version 3.6 (Posada & Crandall 1998) indicated the use of the HKY85 model (Hasegawa *et al.* 1985), with gamma correction. Parsimony and ML trees were constructed under the heuristic search option with 100 random-taxon-addition replicates and tree bisection–reconnection branch swapping, using PAUP*. Node support was assessed on the basis of 1000 bootstrap replicates.

## SYSTEMATICS

Subfamily DYNASTINAE MacLeay, 1819
Tribe PENTODONTINI Mulsant, 1842
Genus *Pentodon* Hope, 1837

### ***Pentodon idiota*** Herbst, 1789

MATERIAL STUDIED. — Greece, Parnassos Mt, 38°33'N, 22°33'E, 400-500 m, VI.2011, 1 ♂. — near Kalambaka, 39°42'N, 21°37'E, 250 m, V.2012, 1 ♂. — near Litochoro, 40°06'N, 22°30'E, 290 m, VI.2010, 1 ♂, coll. B.D.

Tribe PHILEURINI Burmeister, 1842
Genus *Phileurus* Kolbe, 1910

### ***Phileurus valgus*** subsp. ***antillarum*** Prell, 1912

MATERIAL STUDIED. — Guadeloupe, Les Saintes, Terre de Bas, 15°85'N, 61°65'W, 100-150 m, II.2011, 2 ♂♂, coll. B.D.

Tribe ORYCTINI Mulsant, 1842
Genus *Oryctes* Illiger, 1798

### ***Oryctes rhinoceros*** Linnaeus, 1758

MATERIAL STUDIED. — Sulawesi, Batutumonga, 2°55'S, 119°52'E, 800-1000 m, XII.2012, 1 ♂, coll. B.D.

Tribe CYCLOCEPHALINI
Laporte de Castelnau, 1840
Genus *Dyscinetus* Harold, 1869

### ***Dyscinetus picipes*** Burmeister, 1847

MATERIAL STUDIED. — Guadeloupe, Petit Bourg, 16°12'N, 61°36'W, 550-600 m, II.2011, 2 ♂♂, coll. B.D.

### Tribe DYNASTINI MacLeay, 1819
### Genus *Allomyrina* Arrow, 1911

#### ***Allomyrina dichotoma*** Linné, 1771

MATERIAL STUDIED. — Taiwan, private breeding, VIII.2012, 1 ♂, coll. B.D.

### Genus *Chalcosoma* Hope, 1837

#### ***Chalcosoma atlas*** Linnaeus, 1758

MATERIAL STUDIED. — Thailand, private breeding, X.2008, 1 ♂, coll. B.D.

### Genus *Augosoma* Burmeister, 1847

#### ***Augosoma centaurus*** Fabricius, 1775

MATERIAL STUDIED. — Cameroon, private breeding, I.2013, 1 ♂, coll. B.D. Specimens from four other Scarabaeidae sub-families were added to show their chromosomal similarities.

### Subfamily RUTELINAE MacLeay, 1819
### Genus *Anisoplia* Dejean, 1821

#### ***Anisoplia flavipennis*** Brullé, 1832

MATERIAL STUDIED. — Greece, near Ioannina, Greece 39°41N, 20°50'E, 500-600 m, V.2010, 2 ♂♂, coll. B.D.

### Subfamily MELOLONTHINAE Samouelle, 1802
### Genus *Amphimallon* Berthold, 1827

#### ***Amphimallon majale*** Razoumowsky, 1789

Material studied. — France, Bois-le-Roi, 48°27'N, 2°42'E, 90-100 m, VI.2009, 2 ♂♂, coll. B.D.

### Subfamily CETONIINAE Leach, 1815
### Genus *Eudicella* White, 1839

#### ***Eudicella gralli gralli*** Buquet, 1836

MATERIAL STUDIED. — Congo, breeding from Besançon insectarium, XI.2010, 2 ♂♂, coll. B.D.

### Subfamily EUCHIRINAE Hope, 1840
### Genus *Propomacrus* Newman, 1837

#### ***Propomacrus bimucronatus*** Pallas, 1781

MATERIAL STUDIED. — Turkey, 2 ♂♂ specimens, private breeding, II.2009, two males, coll. B.D.

## RESULTS

For making comparisons easier, we will briefly describe and show male karyotypes of species from four sub-families Melolonthidae not belonging to Dynastinae. Then, we will describe the karyotypes of species of Dynastinae belonging to four tribes other than Dynastini, and finally those of species belonging to Dynastini, including *A. centaurus.* All karyotypes were unpublished, but eventual data on chromosomes are indicated.

### MELOLONTHIDAE (DYNASTINAE EXCLUDED)

Anisoplia flavipennis*: 20,XY (Fig. 1A)*
Published data: none. The size of the metacentric/sub-metacentric autosomes is progressively decreasing. The X is a small sub-metacentric and the Y is punctiform. C-bands are small and limited to centromeric regions.

Amphimallon majale*: 20,XY (Fig. 1B)*
Published data: Dutrillaux & Dutrillaux (2012). The size of autosomes is progressively decreasing. They are all meta- or sub-metacentric. Compared to *A. flavipennis*, the centromeres of pairs 4 and 6 are fairly distal and that of pair 1 is more median. C-bands are larger. The X is a small sub-metacentric and the Y is punctiform, but heavily C-banded.

Eudicella gralli gralli*: 20,XY (Fig. 1C)*
Published data: Dutrillaux & Dutrillaux (2012). All autosomes are meta-/sub-metacentric and of progressively decreasing size. The X is a small sub-metacentric and the Y is punctiform. NOR location: X chromosome.

Propomacrus bimucronatus*: 20,XY (Fig. 1D)*
Published data: Dutrillaux & Dutrillaux (2012). The size of autosomes is progressively decreasing, all but pair no. 9 are meta-/sub-metacentric and pair

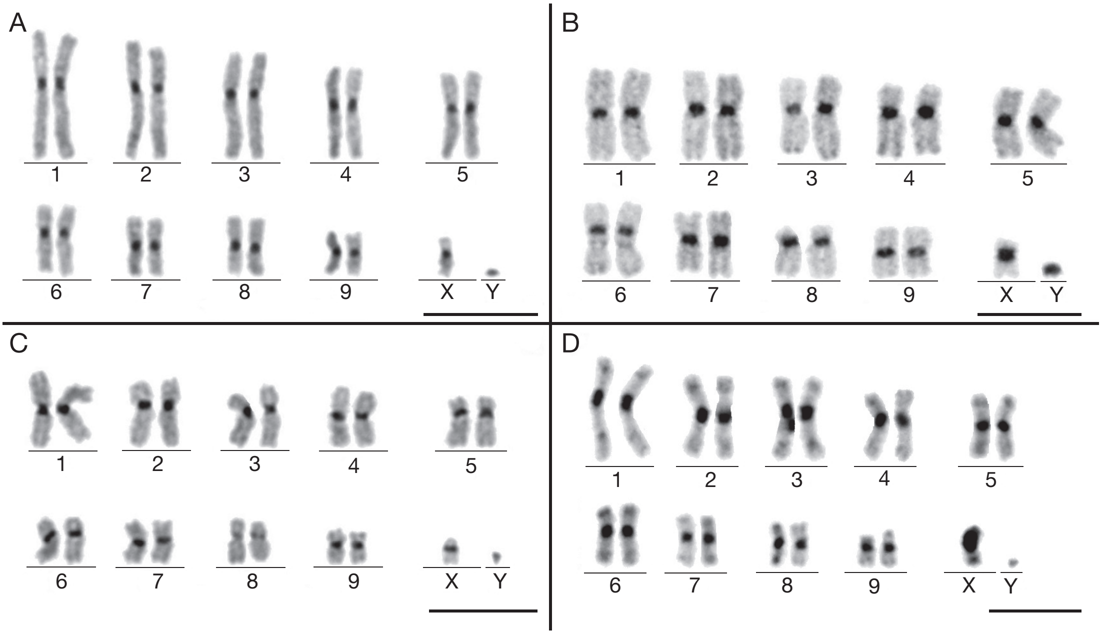

FIG. 1. — C-banded Karyotypes of representative of four Melolonthidae Baraud, 1992 subfamilies: **A**, *Anisoplia flavipennis* Brullé, 1832 (Rutelinae); **B**, *Amphimallon majale* Razoumowsky, 1789 (Melolonthinae); **C**, *Eudicella gralli* Buquet, 1836 (Cetoniinae); **D**, *Propomacrus bimucronatus* Pallas, 1781 (Euchirinae). Scale bars: 10 µm.

no. 9 is almost acrocentric. The X is enlarged by a large C-band, while the Y remained punctiform. There are some small intercalary C-bands on larger autosomes. NOR location: chromosome 9.

DYNASTINAE (DYNASTINI EXCLUDED)

Pentodon idiota*: 20,XY (Fig. 2A)*

Published data: Dutrillaux & Dutrillaux (2012). The size of the meta-/sub-metacentric autosomes is progressively decreasing, but there is a hiatus between pairs 2 and 3 and 5 and 6. The X is a small acrocentric and the Y is a very small punctiform, which can be easily missed. C-banding is very discrete. NOR location: X chromosome.

Phileurus valgus antillarum*: 20,XY (Fig. 2B)*

Published data: Dutrillaux & Dutrillaux (2012). All the autosomes are meta-/sub-metacentric. The size of autosomes 2-8 is progessively decreasing, pair no. 1 is large and pair no. 9 quite small. The X is a small metacentric and the Y is punctiform. NOR location: X chromosome.

Oryctes rhinoceros*: 18,neoXY (Fig. 2C)*

Published data: Yadav *et al.* (1979) and Dasgupta (1977) reported a 20,XYp formula, but no chromosome description. However, we found this karyotype with 18 chromosomes quite original, compared to others. Pairs nos 1 and 2 are sub-metacentric, and all other autosomes are acrocentric (a single euchromatic arm), but look more or less sub-metacentric by the addition of large and variable heterochromatic fragments on their short arms. A large portion of the sub-metacentric X and acrocentric Y undergo synapsis at the pachytene stage and chiasma at the metaphase I of meiosis (not shown) demonstrating its autosomal origin and that the neo-sex chromosomes derive from an X-Y-autosome translocation. This karyotype, close to that of *Oryctes nasicornis* Linnaeus, 1758 (Dutrillaux & Dutrillaux 2009a), only differs from it (neglecting heterochromatin variations) by the inversion of pair no. 8, which is metacentric in *O. nasicornis*. NOR location: small autosome (no. 6).

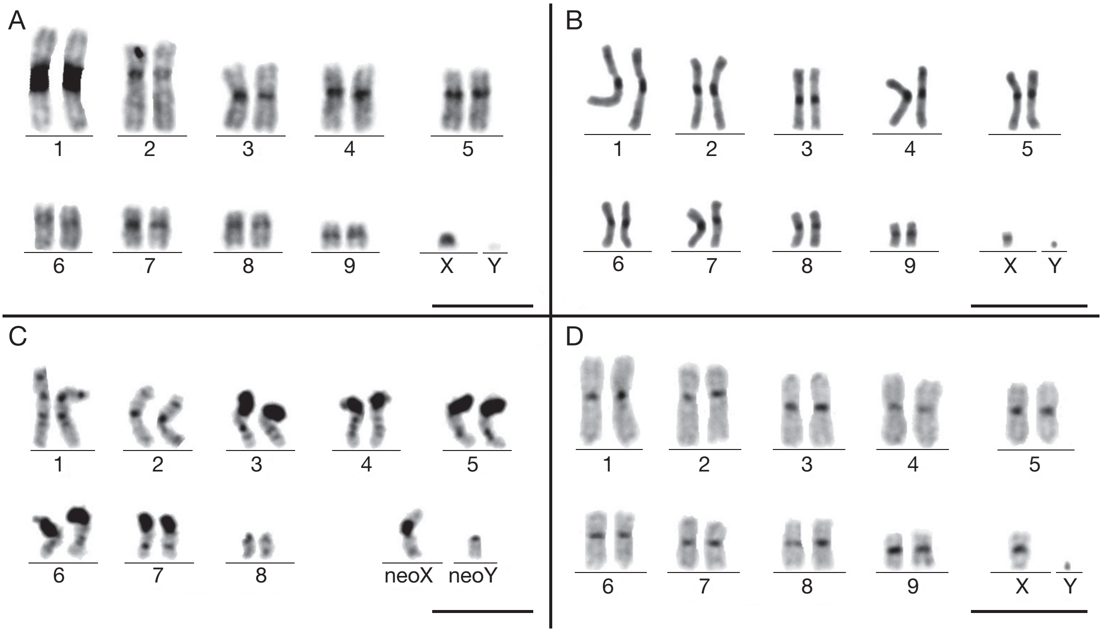

FIG. 2. — C-banded karyotypes of representatives of four Dynastinae tribes: **A**, *Pentodon idiota* Herbst, 1789 (Pentodontini); **B**, *Phileurus valgus antillarum* Prell, 1912 (Phileurini); **C**, *Oryctes rhinoceros* Linnaeus, 1758 (Oryctini), in this karyotype, heterochromatin is very abundant, forming short arms of acrocentrics (dark portions on nos 3-7) and intercalated in euchromatin (small dark bands in all autosomes), heterochromatin is much less abundant in *O. nasicornis* Linnaeus, 1758; **D**, *Discinetus picipes* Burmeister, 1847 (Cyclocephalini). Scale bars: 10 µm.

Dyscinetus picipes*: 20,XY (Fig. 2D)*
Published data: none. Pairs 1,3,5,7 and 9 are almost metacentric and pairs 2, 4, 6 and 8 are sub-metacentric, The X is sub-metacentric and the Y is punctiform. NOR location: X chromosome.

DYNASTINI
Allomyrina dichotoma*: 20,XY (Fig. 3A)*
Published data: Smith & Virkki (1978). The size of the autosomes is less regularly decreasing, which may be due to the amplification of heterochromatin, very abundant and unevenly distributed in centromeric regions. All are meta/sub-metacentric. The sub-metacentric X is also enlarged by heterochromatin. The Y remains punctiform. NOR location: X chromosome.

Chalcosoma atlas*: 20,neoXY (Fig. 3B)*
Published data: Dutrillaux & Dutrillaux (2012). This karyotype is fairly unusual, because the formation of its large and bi-armed neo sex-chromosomes, probably by a double translocation with autosomes, is not accompanied by a reduced number of autosomes. The size of the large chromosomes (nos 1-3) is roughly similar, instead decreasing in size, which suggests that fragments of the two largest of them contributed to form the neoXY by translocation. Heterochromatin is fairly abundant at all centromeric regions. All chromosomes are meta-/sub-metacentric. NOR location: large autosome (no. 3).

Augosoma centaurus*: 18,neoXY (Fig. 3C)*
Published data: none. Pairs nos 1 and 8 are sub-metacentric and all other autosomes are acrocentric. The X is large and sub-metacentric and the Y is acrocentric. Their meiotic behavior, i.e. compaction, synapsis and chiasma formation is typically that of neo-chromosomes formed by X-Y-autosome translocation (not shown). NOR location: small autosome.

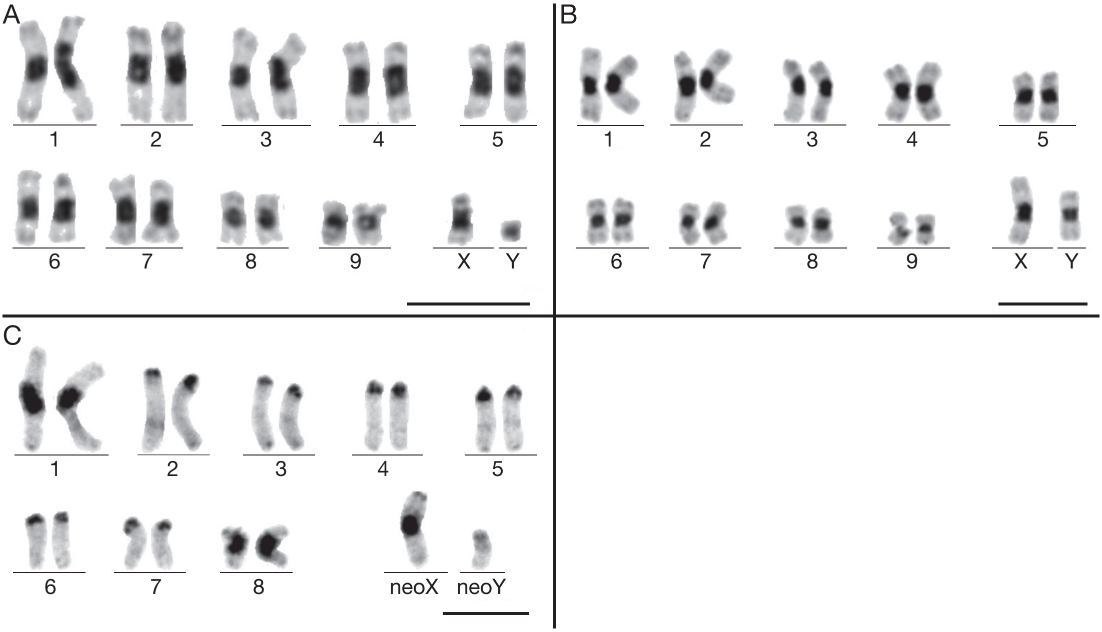

FIG. 3. — C-banded karyotype of three representatives of Dynastini: **A**, *Allomyrina dichotoma* Linné, 1771; **B**, *Chalcosoma atlas* Linnaeus, 1758; **C**, *Augosoma centaurus* Fabricius, 1775. Notice that most chromosomes of *A. centaurus* are acrocentric (entirely heterochromatic [dark] short arms) as in *O. rhinoceros* Linnaeus, 1758 (Fig. 2) and *O. nasicornis* Linnaeus, 1758 (Dutrillaux & Dutrillaux, 2012). Scale bars: 10 µm.

# DISCUSSION

## CHROMOSOME COMPARISONS BETWEEN REPRESENTATIVES OF THE MELOLONTIDAE SUB-FAMILIES

The four 20,XY karyotypes described above exhibit many resemblances. Indeed, they were selected to show that species from four different sub-families, i.e. Rutelinae, Melolonthinae, Cetoniinae and Euchirinae, have fairly similar karyotypes. The question is that of their representation. Except for Euchirinae, these sub-families are composed of several hundreds or even thousands species, of which a limited number was cytogenetically studied. Gathering published (Moura *et al.* 2003; Bione *et al.* 2005; Dutrillaux *et al.* 2008; Dutrillaux & Dutrillaux 2012) and our own unpublished data giving chromosome morphology, we nevertheless came to the conclusion that in each taxonomic group, 20,XY karyotypes with 18 meta-/sub-metacentric autosomes, a small acrocentric or sub-metacentric X, and a punctiform Y are the most frequent. This does not mean that no chromosome changes occurred, but if they did, for a yet unclear reason, they did not drastically change the general configuration of the karyotypes. Robertsonian evolution does not seem to operate, and the rare changes in number, principally decreases, are essentially associated with the presence of neo sex chromosomes as the result of X-Y-autosome translocation. Rutelinae species of the genus *Macraspis* MacLeay, 1819 constitute rare exceptions with 18 autosomes and no neo sex chromosomes (Bione *et al.* 2005; Dutrillaux & Dutrillaux 2009b). As neo sex chromosomes, acrocentric autosomes, which occasionally occur, appear to be rather derivative than ancestral chromosomes, as the consequence of pericentric inversions.

## CHROMOSOME COMPARISON BETWEEN DYNASTINAE SPECIES, DYNASTINI EXCLUDED

Dynastinae are composed of almost 1400 species of which Dynastini is one of the smallest tribes. Published data including chromosome morphologies

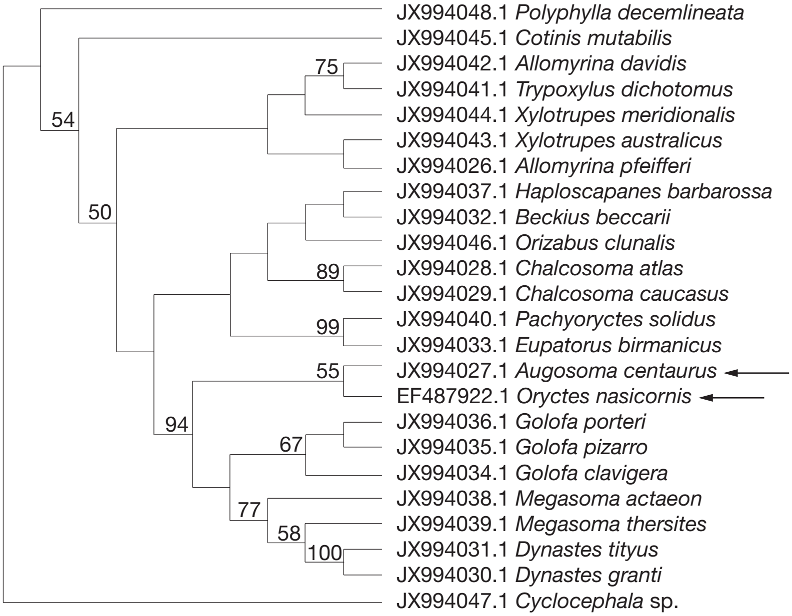

FIG. 4. — Phylogenetic tree resulting from the Parsimony analysis, clustering the 24 of the retrieved sequences from GenBank. The topology of the clusters was similar for the ML tree. Numbers above branches of the major clusters correspond to percentages of Bootstrap values (1000 replications). Only percentages above 50 % are shown. Arrows indicate *A. centaurus* Fabricius, 1775 and *O. nasicornis* Linnaeus, 1758.

are scarce, and limited to some Cyclocephalini (Giannoulis *et al.* 2012), Pentodontini (Vitturi *et al.* 2003) and Oryctini (Vidal & Giacomozzi 1978; Dutrillaux *et al.* 2007; Dutrillaux & Dutrillaux 2009b). Here again, 20,XY karyotypes, with meta-/sub-metacentric autosomes, either acrocentric or sub-metacentric small X and punctiform Y are observed in all tribes, as reported here in *P. idiota* (Pentodontini), *P. valgus antillarum* (Phileurini) and *D. picipes* (Cyclocephalini). The NOR is located on the X chromosome. According to the parsimony principle, this means that such karyotypes resemble ancestral ones, not or only slightly modified. Compared to them, the karyotype of *O. rhinoceros* looks very atypical, with 16 autosomes, including 12 acrocentrics carrying variable amounts of heterochromatin on their short arms and neo sex chromosomes. The NOR is located on a small autosome. Compared to that of *O. nasicornis* (Dutrillaux & Dutrillaux 2009), the karyotype of *O. rhinoceros* differs by a single change: pair no. 8 is acrocentric in *O. rhinoceros* and metacentric in *O. nasicornis* (we neglect usual variations of heterochromatin). Thus, the karyotype of *O. nasicornis* derives from the ancestral one by a minimum of six inversions forming its acrocentrics and one translocation forming its neo sex chromosomes and another one displacing the NOR, and that of *O. rhinoceros* by an additional inversion (Fig. 4). Five species of *Cyclocephala* Dejean, 1821 (Cyclocephalini) were studied and their presumed ancestral karyotype was reconstructed (Dutrillaux *et al.* 2007; Giannoulis *et al.* 2012). Although also composed of 18 autosomes and XY, this karyotype differed from the other Dynastinae species, including *D. picipes* (Cyclocephalini), by the larger size of pair no. 1 and the less median position of the centromeres of chromosomes 1-5. Thus, at least one translocation formed pair no. 1 and four pericentric inversions modified pairs nos 2-5.

### CHROMOSOME COMPARISON BETWEEN DYNASTINI SPECIES

In addition to the karyotype of the two Australasian Dynastini species, *A. dichotoma* and *C. atlas,* described above, we studied that of *Xylotrupes gideon* s.l. Guérin-Méneville, 1830 (Dutrillaux & Dutrillaux 2013: 37-42): it differs from that of *A. dichotoma* by its acrocentric chromosome 9. The karyotype of *C. atlas* differs from that of *X. gideon* s.l. by the presence of large metacentric neo sex chromosomes, the smaller size of its chromosomes nos 1 and 2 and its chromosome 9 metacentric. Among neotropical species, *Megasoma actaeon* Linnaeus, 1758 has a karyotype (Dutrillaux *et al.* 2007) fairly similar to that of *A. dichotoma*, when heterochromatin, much more abundant in the latter, is neglected. The karyotypes of *Dynastes satanas* Moser, 1909 and *D. neptunus* Quensel, 1817 differ by their chromosome 8, acrocentric. Those of *Dynastes hercules*, *D. tityus* Linnaeus, 1758 and *D. granti* Horn, 1870 underwent a translocation of this acrocentric onto the sex chromosomes, forming neo sex chromosomes (Dutrillaux & Dutrillaux 2013). On the whole, the karyotypes of Neotropical and Australasian Dynastini species are not much different. At contrast, the karyotype of the African species *A. centaurus,* with six pairs of acrocentric autosomes, autosomal NOR and neo sex chromosomes, appears to be highly derived.

### CHROMOSOME COMPARISON BETWEEN ALL SPECIES

Fairly similar karyotypes are found in most representatives of all the sub-families of Melolonthidae considered here, whatever their geographical origin. Such 20,XY karyotypes, with 18 meta/sub-metacentric autosomes, also frequently found in other taxonomic groups among Polyphagan beetles, are likely to represent an ancestral status, at a much higher taxonomic level, such as that of the Polyphagan ancestors. The regular size decrease observed in most species suggests that neither reciprocal nor Robertsonian translocations occurred frequently. At contrast, the positions of the centromeres vary, making a given pair more or less metacentric, as probable consequence of pericentric inversions. Unfortunately, in the absence of accurate chromosome identification, it is not possible to give more precision about the exact morphology of ancestral chromosomes individually. Acrocentric autosomes are sporadically observed. Their rare occurrence and the involvement of chromosomes of different sizes indicate that the rearrangements originating acrocentrics independently occurred in various systematic groups. In this inter-specific comparison, two karyotypes, those of *O. rhinoceros* and *A. centaurus*, are unusual because they are mainly composed of acrocentric autosomes. They also have similar neo sex chromosomes, which differ from those of *Dynastes* and *Chalcosoma* species by their size and morphology. On the whole, the karyotypes of *O. rhinoceros* and *A. centaurus* may differ from each other by two pairs (nos 2 and 8) only, when heterochromatin variations are neglected. Furthermore, the karyotype of *O. rhinoceros* differs from that of *O. nasicornis* by its pair no. 8, metacentric in the latter (Dutrillaux & Dutrillaux 2012), as in *A. centaurus*. Thus, the karyotypes of *A. centaurus* and *O. nasicornis* may differ by a single chromosome rearrangement. Finally, the two *Oryctes* species and *A. centaurus* are the only species of Dynastinae studied with a NOR located on a small autosome. Thus, either two series of rare rearrangements involving the same chromosomes independently occurred, or genera *Oryctes* and *Augosoma* are phylogenetically very close to each other. This does not fit with the generally accepted position of the two genera in two different tribes, Dynastini for *Augosoma* and Oryctini for *Oryctes* (Endrödi 1985).

### THE AMBIGUOUS SYSTEMATIC POSITION OF *AUGOSOMA CENTAURUS*

The classification of *A. centaurus* among Dynastini was based, as usual, on morphological characters and more precisely here, on exaggerated characters of the males, i.e. large body size, very long frontal and pronotal horns and long fore legs. It is true that males of *A. centaurus* share these characters with the males of most other Dynastini species. However, the large amplitude of these characters is facultative, and not limited to Dynastini. Among Oryctini for instance, some species, as *Oryctes gigas* Castelnau, 1840, are very large, and the males of many species have long frontal and pronotal horns. This means that the morphological difference between males of Oryctini and Dynastini is not so clear cut and it may be hazardous to base their systematic classification mainly on their exaggerated characters.

The resulting sexual dimorphism essentially has an epigenetic determinism, hormonally triggered during metamorphosis and is particularly dependent on environmental factors (Emlen & Nijhout 1999; Emlen *et al.* 2006). This is responsible for a strong polymorphism of the males which may lead to misinterpretations in inter-specific comparisons. Female characters are less spectacular and also much less variable. They are rarely considered to establish systematic classification including for Dynastinae. In many Dynastini females, the pronotum is without reliefs. Its whole surface is wrinkled and that of elytra punctuate and setose. These characters are not found in *Oryctes* females, in which the elytra are shining and smooth, while the pronotum is smooth and shining in its posterior part and punctuate in its anterior part, which exhibits a cavity. *Oryctes* females also have a small frontal horn or plate. All these characters are precisely found in *A. centaurus* females. Thus, in *A. centaurus*, males look like Dynastini and females look like Oryctini, and more precisely like *Oryctes* females. It could be proposed that the position of *A. centaurus* is intermediate between Dynastini and Oryctini, which would fit with its African localization as a relict species of a tribe of a Gondwanan origin. However, our chromosomal data show that the genera *Augosoma* and *Oryctes* shared many chromosomal changes, not shared by other Dynastinae. This reflects a long common evolution, not compatible with an intermediate position between two groups of Dynastinae and indicates they are monophyletic.

### CONFIRMATION OF THE *ORYCTES-AUGOSOMA* PROXIMITY BY DNA SEQUENCE DATA

The similarities between the karyotypes of *Oryctes* and *Augosoma* do not however constitute an absolute demonstration of their phylogenetic proximity, in the absence of molecular identification of their chromosomes. Karyotypes of other Scarabaeoids, belonging to Dynastinae (*Cyclocephala tridentata* Fabricius, 1801), Cetoniinae (*Goliathus goliathus* Drury, 1770; *Osmoderma lassalle*i Baraud & Tauzin, 1991), Scarabaeinae (*Bubas bison* Linnaeus, 1767 and *Copris hispanus* Linnaeus, 1764) and Geotrupidae (*Geotrupes stercorarius* Linnaeus, 1764) among others, are mainly composed of acrocentrics (Wilson & Angus 2004; Angus *et al.* 2007; Dutrillaux *et al.* 2007; Dutrillaux & Dutrillaux 2012), but the phenotypic differences are sufficient to consider that purely convergent chromosome rearrangements formed these acrocentrics. However, this is not the case for *Oryctes* and *Augosoma* species which share many phenotypic characters, but a molecular confirmation was needed. Two sets of published data on DNA sequencing can be used to strengthen our interpretation: a large survey which includes *O. nasicornis* (Hunt *et al.* 2007) and a study of Dynastini which includes *A. centaurus* (Rowland & Miller 2012). In both studies, sequences of the 16S rRNA gene were also used to assess phylogenetic reconstruction between species. To test our hypothesis the 16S rRNA sequence of *O. nasicornis* (Hunt *et al.* 2007) was analysed together with those of Dynastini which includes *A. centaurus* (Rowland & Miller 2012). Phylogenetic reconstruction confirmed the proximity of *A. centaurus* and *O. nasicornis* (Fig. 4): they are monophyletic and well separated from other Dynastini, which fits with the chromosome data. Interestingly, they are placed between Australasian and American Dynastini species. Such results can be variously interpreted. A first interpretation would consist of leaving *Augosoma* within Dynastini, but then, the genus *Oryctes* should be included too. A second interpretation would consist of placing *Augosoma* among Oryctini, but their multiple shared chromosome rearrangements separate *Oryctes* and *Augosoma* from other genera of Oryctini, such as *Strategus* Hope, 1837, *Enema* Hope, 1837 and *Cyphonistes* Burmeister, 1847, which have no acrocentrics in their karyotype (Vidal & Giacomozzi 1978; Dutrillaux *et al.* 2007 & unpublished). Thus, the only certitude is the genetic proximity of the genera *Augosoma* and *Oryctes*, hardly compatible with their classification into two different tribes, and their separation from the other Dynastinae genera studied so far. Because of this proximity, the change the most compatible with genetics would be to suppress the distinction between Oryctini and Dynastini by including former Oryctini species into the tribe Dynastini, but this conclusion must be validated by the study of more species, in particular from other genera of Oryctini.

### BIO-GEOGRAPHICAL CONSIDERATIONS

It is generally admitted that Dynastinae originated prior to the dislocation of the Pangaea, i.e. more than 200 million years ago, which would explain their present distribution (Endrödi 1985). The species distribution of the small tribe Dynastini fits with this possible origin, although the presence of a single species, *A. centaurus*, in Africa looks surprising. This lack of expansion in Africa was explained by climatic changes, to which thermophilous Dynastini species seem particularly sensitive. However, if *A. centaurus* is brought near to *Oryctes*, as the above results suggest, then, it would join a taxonomic group well developed in Africa, which would be more coherent. However, if *Augosoma* is placed within Oryctini, then, the tribe Dynastini becomes purely dichotomic and the hypothesis of a Gondwanan origin would not fit so well with an American-Australasian link without Africa in between. It is noteworthy that such particularity would not be limited to Dynastini, because 30 genera or sub-genera comprising 700 species of crane flies (Diptera: Tipulomorpha) link South America and Australasia, without any other species in Africa (Ribeiro & Eterovic 2011). These authors challenge their Gondwanan origin and discuss a possible shared history in the South Pacific. Our data might be interpreted similarly. However, if Oryctini species, which are largely distributed in Africa and Madagascar, as well as in Americas, are grouped with Dynastini, then their Gondwanan origin becomes strongly supported.

## CONCLUSION

Both chromosomal and DNA sequence data indicate that *Augosoma* (Dynastini) is much closer to *Oryctes* (Oryctini) genus than to any other Dynastini genus, which imposes a revision of their taxonomic position. The simplest one would consist in placing the genus *Augosoma* within Oryctini, but it might be wiser to enlarge genetic analyses to reconsider all Oryctini-Dynastini relationships, whose separation into two different tribes seems artificial.

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