

A Chromosomal Analysis of Eight Species of *Melinopterus* (Coleoptera, Scarabaeidae: Aphodiinae)

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Abstract—The chromosomes of eight species of *Melinopterus* Mulsant, 1842 are described and illustrated, five of them, *M. punctatosulcatus* (Sturm, 1805), *M. maroccanus* Rößner, 2018, *M. villarreali* (Baraud, 1975), *M. abeillei* (Sietti, 1903) and *M. tingens* (Reitter, 1892), for the first time. *Melinopterus villarreali* is shown to be a valid species, distinct from *M. sphacelatus* (Panzer, 1798). All the species have distinctive karyotypes comprising $2n = 18 + Xy$ (♂), $2n = 18 + XX$ (♀), but those of female *M. villarreali* and *M. tingens* are difficult to distinguish because, although their X chromosomes are of different sizes, it is difficult to pick out the correct chromosome.

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INTRODUCTION

I first met Gleb Sergeevich Medvedev in 1969–1970, when I worked in the Zoological Institute, Leningrad, and found him to be one of the kindest and friendliest people I have ever met. A further meeting in 1982 only reinforced this impression. It was a pleasure to offer papers as a tribute to Gleb Sergeevich in 2006 in celebration of his 75th birthday (Beauchamp and Angus, 2006; Wilson and Angus, 2006) and now a further pleasure and a privilege to be invited to offer this as a further tribute on the 10th anniversary of his death.

Melinopterus Mulsant, formerly placed as a subgenus of *Aphodius* Hellwig but elevated to generic status in the revised Catalogue of Palaearctic Coleoptera (Dellacasa et al., 2016), is a particularly interesting group for chromosomal analysis because of the variety of form of its sex chromosomes, the well-marked C-banding of all the chromosomes, and the level of variation both between and within species (Wilson and Angus, 2003). Wilson and Angus studied three British species, and the present account increases the number of studied species to eight, all with distinctive karyotypes and all with karyotype formula $2n = 18 + Xy$ (male), XX (female).

MATERIALS AND METHODS

Details of the species studied are given in the table. In all cases the numbers of specimens listed are those from which successful chromosome preparations have been obtained.

Chromosome preparations were obtained from mid gut of adult beetles. Beetles are injected with 0.1% colchicine solution in insect saline (0.75% NaCl in distilled water buffered to pH 6.8 with Sørensen's phosphate buffer) and left for about 12.5 min. They are then transferred to a 0.48% ($1/2$ -isotonic) solution of KCl at pH 6.8 in individual solid watch glasses, their abdomens detached and the mid guts removed and left in the solution. The rest of the beetle is removed, killed by immersion in boiling water, and mounted on card as a voucher. After 12.5 min. the guts are transferred to fixative (3 parts absolute ethanol to 1 part glacial acetic acid), again in solid watch glasses. The fixative is changed twice and the guts are then left for 1 hour, with the watch glasses covered to prevent water being absorbed from the air. For chromosome preparations small pieces of tissue are taken with fine forceps and placed on clean dry slides, cells were disaggregated in a small drop of 45% acetic acid, with the tissue torn apart with fine pins as necessary. Next, one drop of fixative is pi-

Material studied

<i>Melinopterus</i> species	Locality	Number of specimens
* <i>M. sphacelatus</i> (Panzer, 1798)	Netherlands, Zuid-Holland: Leidschendam, Vlietland, 52.1005°N, 4.414°E UK, Surrey: Bookham Common, 51.283°N, 0.3958°W Spain, Madrid: Velilla de San Antonio, 40.36961°N, 3.4868°E	1 ♂ 1 ♂ 1 ♂
<i>M. punctatosulcatus</i> (Sturm, 1805)	UK, Kent: Sandwich, 51.2669°N, 1.3674°E	3 ♂, 2 ♀
<i>M. maroccanus</i> Rößner, 2018	Morocco, Moyen Atlas: by Lac Afenourrir near Ifrane, 33.285°N, 5.252°W	1 ♂, 3 ♀
<i>M. villarreali</i> (Baraud, 1975) stat. res.	Spain, Cadiz: Venta de Facinas, 36.128°N, 5.711°W Morocco, Rif: Talassemntane National Park, 35.165°N, 5.186°W	3 ♂, 1 ♀ 1 ♂
* <i>M. prodromus</i> (Brahm, 1790)	UK, Surrey: Bookham Common, 51.283°N, 0.3958°W	1 ♂
* <i>M. consputus</i> (Creuzter, 1799)	UK, Kent: Lydden, 51.161°N, 1.237°E Italy, Sardinia, Oristano: S'Arenarzu, 40.004°N, 8.413°E	1 ♂ 1 ♀
<i>M. abeillei</i> (Sietti, 1903)	Spain, Malaga, La Saucedá, 36.128°N, 5.585°W	4 ♂, 1 ♀
<i>M. tingens</i> (Reitter, 1892)	Spain, Cadiz: San Roque, 36.236°N, 5.348°W; Venta de Facinas, 36.128°N, 5.711°W	5 ♂, 2 ♀ 2 ♀

*Only material figured here is listed. For full details of these species, see Wilson and Angus, 2003.

petted onto the cell suspension. This will cause the drop to spread over the slide as a thin film. The spreading film can be guided by tilting the slide. Sides are dried horizontally. After at least 1 hour, they are stained with 0.5% (approximately) Giemsa solution at pH 6.8.

Chromosomes were photographed under oil-immersion (X100 objective) onto high-contrast microfilm. Photographs were printed at X 3000, then scanned into a computer and further processed using Adobe Photoshop.

For C-banding the immersion oil used for photographing the preparations is removed by immersion in Coplin jars containing xylene (2 changes) and absolute ethanol. They are then dried vertically. C-banding is done by immersing the slides in saturated barium hydroxide at room temperature (about 22°C) for about 4 minutes, followed by 1 hour in 2X SSC (Salt-Sodium Citrate: 0.3 M NaCl + 0.03 M trisodium citrate) at 60°C. The C-banding protocol can be repeated if initial results are inadequate.

Beetles were photographed using a Leica MZ 125 stereo microscope equipped with a Cannon DSLR camera. Beetles were illuminated by two IKEA

“Jansjö” LED lights with a ring of tracing paper to act as a diffuser. Images were stacked using Helicon Focus software.

RESULTS

The *M. sphacelatus* Group of Species

These species are characterised by having stria eight of the elytra almost as long as stria seven basally (Figs. 3a–3d), as against being distinctly shorter, with stria seven extending basally by about four punctures more than stria eight (Figs. 3e, 3f). The parameres of *M. sphacelatus* group species have the soft apical section only slightly widened, giving an almost match-stick like appearance in dorsal view, while it is more distinctly flared in most of the others; see the figures 164–176 in Baraud (1992).

M. sphacelatus

This is one of the commonest British Aphodiines. Wilson and Angus (2003) note the presence of B-chromosomes in both English and Dutch material, and show that these chromosomes are partially heterochromatic, darkly stained following mild C-banding treatments,

but after stronger treatments, apart from the small centromere, completely unstained even though the C-bands of all the chromosomes, and the heterochromatic long arm of the X chromosomes, are strongly stained. The chromosomes of the Spanish male figured here (Figs. 1b, 1c) show no difference from those of English and Dutch specimens (Fig. 1a).

M. punctatosulcatus

This species was until recently known in England only from material dating from the beginning of the twentieth century, all from east Kent, and was presumed to be extinct in Britain. However, in Spring 2018 Darren Mann (Oxford) rediscovered it in the Sandwich area of east Kent. I collected it there in May 2019, and the chromosomes figured here (Figs. 2d, 2e) are from this material. As in other species of the genus, the karyotype comprises nine pairs of autosomes and sex chromosomes which are Xy (male) and XX (female). The chromosomes differ from those of *M. sphacelatus* in that chromosome 1 is clearly submetacentric (all the autosomes are metacentric in *M. sphacelatus*) and all are quite strongly C-banded (pairs 1–3 have small, often indistinct, C-bands in *M. sphacelatus*). Pairs 6 and 7 have particularly extensive centromeric C-bands. The form and C-banding of both the X and y chromosomes are similar to those of *M. sphacelatus*.

M. maroccanus

This species was originally considered by Rößner (2018) to be a subspecies of *M. sphacelatus*, but when I showed him my chromosome preparations in October 2018, he realised that it had to be a separate species. His revision was by that time in an advanced state of production, but he was able to add a paragraph upgrading it to species rank, and also to show karyotypes of both *A. sphacelatus* (from Wilson and Angus, 2003) and *M. maroccanus* (my unpublished material).

The most striking difference between the two species is in the form of the sex chromosomes, which in *M. maroccanus* lack heterochromatin except for very small centromeric C-bands. The X chromosome is a small acrocentric, possibly homologous with the euchromatic short arm of the *M. sphacelatus* X, and the y is small, little more than a dot. The autosomes are also completely different in the two species – in *M. maroccanus* there is a sharp decrease in size between pairs 6 and

7 whereas in *M. sphacelatus* there is a more gradual decrease in size along the karyotypes. In addition, the C-banding of the autosomes is strikingly different between the two species (Figs. 1a, 1c, 1g).

M. villarreali stat. rest.

M. villarreali was treated as a synonym of *M. sphacelatus* by Rößner (2018) as the character used to separate the two forms, the paler clypeal patches (Figs. 3g–3i), was variable and sometimes indistinct, and beetles with and without the patches quite often occurred together.

M. villarreali was a species I had been looking for in Spain for some years, and I finally found it in November 2018. The chromosomes (Figs. 1h, 3i) turned out to be very clearly not *M. sphacelatus* as, even at a glance, they lacked the very distinctive sex chromosomes of that species. Not only that, but they matched those of a *Melinopterus* I had taken in Rif mountains of Morocco in April 2018 (Figs. 1j, 1k). I had sent this beetle, after I had obtained its chromosomes, to Jason Mate in Madrid for his DNA research, but had kept as a voucher the abdomen and aedeagus, mounted on a card. The aedeagus confirmed that this was a *sphacelatus*-group species, and the chromosomes and locality confirmed that this too was *M. villarreali*. The sex chromosomes of *M. villarreali* are unusual for a *Melinopterus*. The X chromosome is a medium-sized metacentric with a moderate centromeric C-band, comparable with autosomes 4 or 5. The y chromosome is a very small, often dot-like acrocentric with a tiny C-band (Fig. 1i). The autosomes as a whole are similar to those of *M. sphacelatus*, but the C-bands of pairs 1–3 are larger.

The Others

The remaining species in this paper are placed by Rößner (2018) in the *M. prodromus*, *M. consputus* and *M. pubescens* (Sturm, 1800) species groups.

M. prodromus

This is one of the commonest species of the genus and was discussed in detail by Wilson and Angus (2003). The karyotype of a British specimen is shown in Figs. 2a, 2b. The X chromosome has a heterochromatic long arm and the y chromosome is a small submetacentric with a heavy centromeric C-band. Auto-



Fig. 1. Karyotypes of *Melinopterus sphacelatus*-group species, from mitotic mid-gut chromosomes of adult beetles: (a–c) *M. sphacelatus* [(a) male, C-banded, Netherlands; (b, c) female, Madrid [(b) Giemsa stained, (c) the same nucleus C-banded]]; (d, e) *M. punctatosulcatus*, male, Sandwich [(d) Giemsa stained, (e) nucleus from the same specimen, C-banded]; (f, g) *M. maroccanus*, male, Moyen Atlas [(f) Giemsa stained, (g) the same nucleus, C-banded]; (h–k) *M. villarreali*, males [(h, i) Venta de Facinas, the same nucleus Giemsa stained and C-banded, (j, k), Rif, the same nucleus Giemsa stained and C-banded]. Scale bar 5 μ m.



Fig. 2. Karyotypes of *Melinopterus* species, not *sphacelatus*-group, from mitotic mid-gut chromosomes of adult beetles: (a, b) *M. prodromus* male with one B-chromosome, from Bookham Common [(a) Giemsa stained, (b) the same nucleus C-banded]; (c, d) *M. consputus*, C-banded [(c) male from Lydden, (d) female from Sardinia]; (e–h) *M. abeillei* La Saucedá [(e, f) male: (e) Giemsa stained, (f) the same nucleus C-banded], (g, h) females [(g) Giemsa stained, (h) C-banded]; (i–k) *M. tingens*, males, San Roque [(i) Giemsa stained, (j) the same nucleus C-banded, (k) another nucleus, C-banded with the bands very clear, but lacking two chromosomes indicated by large dots]. Scale bar 5 μm.

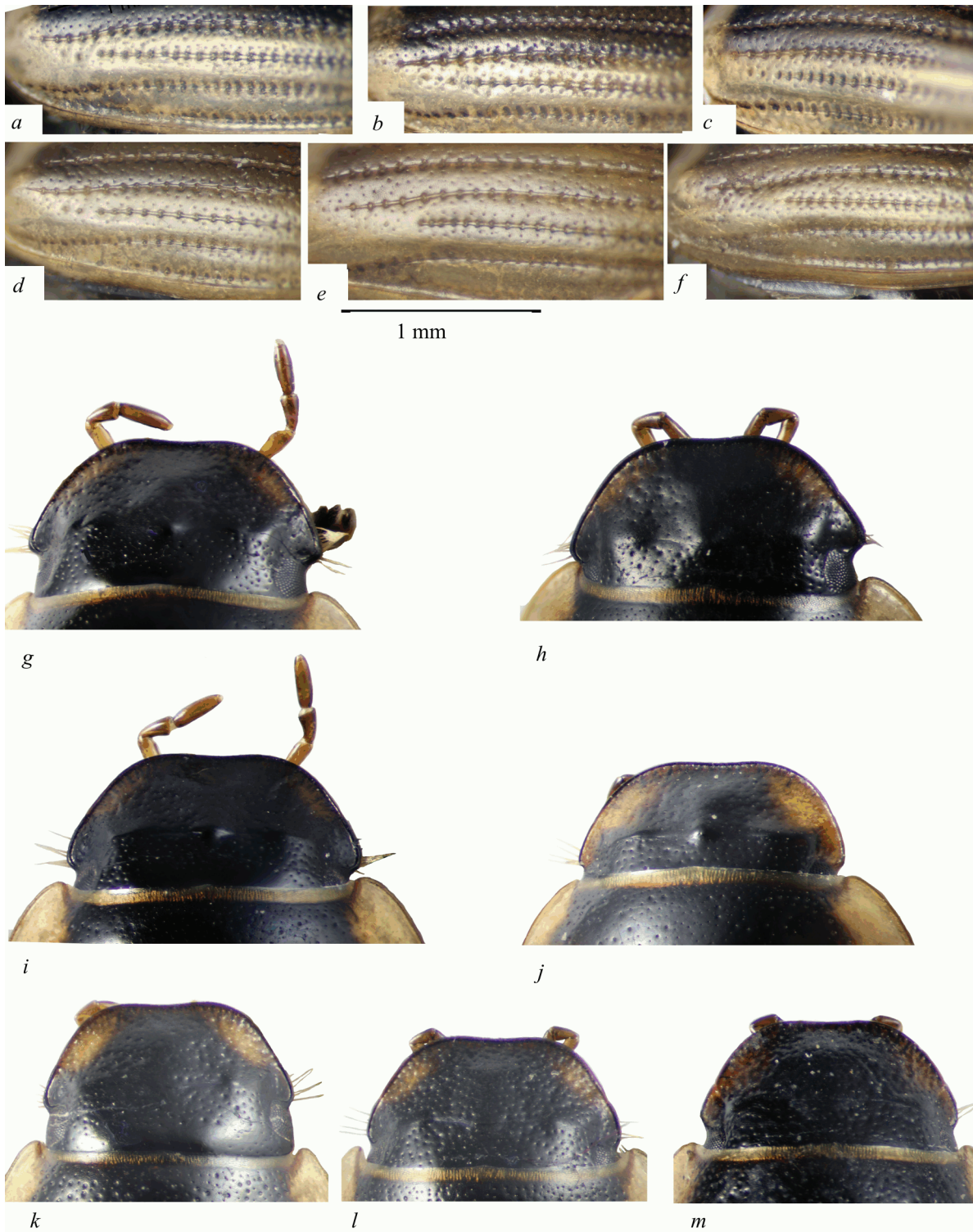


Fig. 3. *Melinopterus* spp., (a–f) elytra showing striae 7 and 8, (g–m) heads showing the paler clypeal patches: (a, g) *M. villarreali* male 3, 29.XI.2018; (b, h) *M. villarreali* male 1, 30.XI.2018; (c, i) *M. villarreali* male 1, 3.XII.2018; (d, k) *M. villarreali* female 3, 30.XI.2018; (e, l) *M. tingens*, Venta de Facinas, female 2, 30.XI.2018; (f, m), *M. tingens*, San Roque, female 7, 23.II.2009; (j) *M. tingens*, San Roque, male 7, 11.XI.2006. Scale bar 1 mm. Note that the elytra shown in a–f were photographed with their anterior ends deflected downwards by about 15° as this gave the clearest display of the striae. The images were then rotated with Photoshop, to show the striae horizontally. This means that they are somewhat foreshortened, but does not affect the appearance of the basal parts of striae 7 and 8.

some 5 is polymorphic for the presence or absence of C-band on its short arm. One B-chromosome may be present, this being acrocentric and slightly longer than the X chromosome and heterochromatic throughout, though the centromeric C-band is darker than the rest of the chromosome.

M. consputus

In Britain this species is confined to the south, mostly on chalk grassland where it feeds mainly on sheep dung. The X chromosome has a heterochromatic long arm and the y is acrocentric with a heavy centromeric C-band (Fig. , This species has a high degree of polymorphism in its C-bands and in the presence or absence of a terminal satellite on the X chromosome. The polymorphism is analysed in detail by Wilson and Angus (2003). Fig. 2d shows a C-banded karyotype from a Sardinian female. This specimen shows no polymorphism and its karyotype is matched by British material.

M. abeillei

Male and female karyotypes, Giemsa-stained and C-banded, are shown in Figs. 2e–2h. Autosome pairs 1–4, 8 and 9 are metacentric, 5 and 7 are submetacentric, while pair 6 is almost acrocentric with heterochromatic short arm. Centromeric C-bands are present on all the autosomes and there are no distinct steps in the progressive reduction of autosome lengths along the karyotype. The X chromosome has a euchromatic short arm and a heterochromatic long arm. The y chromosome appears to be metacentric and entirely heterochromatic, and is slightly longer than the X chromosome

M. tingens

Karyotypes of males are shown in Fig. 2i (Giemsa stained) and Figs. 2j, 2k (C-banded), and a C-banded karyotype from a female is shown in Fig. 4d. All the autosomes, and the X chromosome, are metacentric with

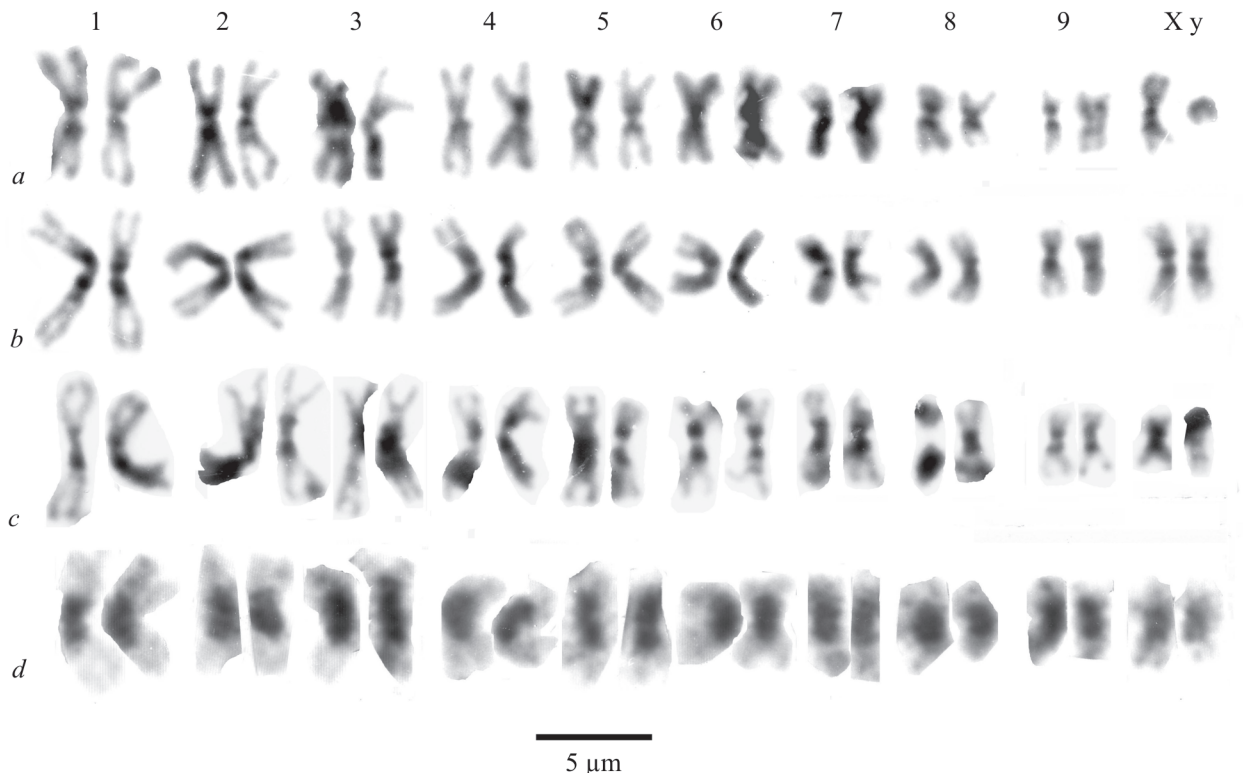


Fig. 4. *Melinopterus villarreali* and *M. tingens*, C-banded karyotypes from mid-gut chromosomes of adults, to show identification of females: (a, b) *M. villarreali* [(a) male 3, 29.XI.2018, (b) female 3, 30.XI.2018]; (c, d) *M. tingens* females [(c) 2, 30.XI.2018, (d) San Roque, 7, 23.II.2009]. Scale bar 5 μ m.

heavy centromeric C-bands. The X chromosome is slightly shorter than the shortest autosome pair (pair 9), unless a secondary constriction is open in one of its arms (Figs. 2*i*, 2*j*). The y chromosome is submetacentric, about two-thirds the length of the X chromosome without the open secondary constriction, and with a distinct centromeric C-band.

M. tingens is one of the species with pale clypeal patches. The normal appearance of the head is shown in Fig. 3*j*. Baraud (1992) notes the occurrence of specimens with the clypeus entirely dark, but only in Gironde (France), where it is not very common and occurs along with the typical form. Rößner mentions that very rarely the head is entirely black, and refers to Baraud's record. I have taken occasional specimens (male and female) at San Roque (Spain) in which the head, examined in the field with a 20× hand lens, appears entirely black. The head of one of these females is shown in Fig. 3*m*, and under the intense illumination of my photographic set-up, shows dark reddish-brown patches comparable with those of my *M. abeillei* males (Figs. 3*g*–3*i*). The identity of the *M. tingens* female is confirmed by its chromosomes (Fig. 4*d*).

The fact that *M. tingens* and *M. abeillei* have X chromosomes, albeit of different sizes, which resemble autosomes in their general form and C-banding poses problems for the identification of females. Eckehard Rößner (pers. comm.) has warned me that the development of elytral striae seven and eight in *M. tingens* can be variable and is thus not an entirely reliable character for recognising that species. I have two females from Venta de Facinas which illustrate the problem. Female 2, 30.XI.2018 (the numbering of the specimens refers to their chromosome preparations), based on its elytral striae (Fig. 3*e*) and its karyotype (Fig. 4*c*) appears to be *M. tingens* although its poorly developed pale clypeal patches (Fig. 3*l*) are more like in some *M. villarreali*. Female 3, 30.XI.2018, on the other hand, has its elytral striae (Fig. 3*d*) of the *M. villarreali* pattern and its chromosomes (Fig. 4*b*) match those of male *M. villarreali* (Fig. 4*a*).

DISCUSSION

The increase in the number of chromosomally analysed species from three to eight has reinforced the idea that their karyotypes are distinctive and species-specific. Of the five species added to the list, two,

M. punctatosulcatus and *M. abeillei*, have the X and y chromosomes with heterochromatic long arms (the y apparently entirely heterochromatic and metacentric in *M. abeillei*), one (*M. maroccanus*) has a small acrocentric X chromosome which could be homologous with the short arms of the X chromosomes of *M. sphacelatus*, *punctatosulcatus* and *abeillei*, and two, *M. villarreali* and *M. tingens*, have metacentric X chromosomes with heterochromatin confined to the centromeric C-bands. It is not possible to say which type of X chromosome is plesiomorphic in *Melinopterus*, and the main feature seems to be that the form of the X chromosome is species-specific and not an indication of particularly close relationship between any individual species. None of the newly added species has been found to show chromosome polymorphism, but that may simply be a reflection of more restricted sampling. The problems associated with identification of female *M. villarreali* and *M. tingens* have already been mentioned. Part of the trouble is a shortage of high-quality karyotypes. Rößner lists localities from where he has seen mixed populations of pale-spotted and uniformly dark headed *M. sphacelatus* (in his interpretation). The most useful ones are from Portugal, and Rößner tells me that this material was collected by Tristão Branco (Porto). Tristão Branco has kindly sent me details of his localities and I hope to visit some of them in November.

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It is a pleasure to thank all who have helped me in the production of this paper. Eckehard Rößner identified my *M. maroccanus* and confirmed my identifications of *M. tingens* and *M. abeillei* and has been consistently helpful and informative. Jason Mate sent me material and accompanied me on several enjoyable Spanish collecting trips. Tristão Branco has given me detailed information about his Algarve collecting sites, and here in England Darren Mann and Ron Carr gave me details of the localities for *M. punctatosulcatus* and Ron and I together collected the species in May this year. My daughter Lizzie (Dr E. M. Angus) has accompanied me on a number of Spanish trips, always adding greatly to the enjoyment and productivity. Finally I thank Max Barclay and the Natural History Museum in London for my continuing position as a Scientific Associate and the use of the Museum's facilities.

ADDITIONAL INFORMATION

This article was originally submitted by the author in English and is published here for the first time.

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