CHROMOSOMAL AND AEDEAGAL DISTINCTION BETWEEN *APHODIUS (LABARRUS) LIVIDUS* OLIVIER, 1789 AND *A. (L.) PSEUDOLIVIDUS* BALTHASAR, 1941 (COL., SCARABAEIDAE, APHODIINAE)

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ABSTRACT

The karyotypes of *Aphodius lividus* (from Greece and Cyprus) and *A. pseudolividus* (from the Dominican Republic and South Africa) are illustrated. They show differences in the form and relative sizes of both the autosomes and the sex chromosomes inconsistent with their belonging to the same species. In addition, South African *A. pseudolividus* has a heterochromatic block on the X chromosome, which is absent from the Dominican material. However, the karyotypes of the *A. pseudolividus* from the two areas are in complete agreement in all other features found and there appears to be no reason not to regard the Dominican Republic and South African material as conspecific. The aedeagophores of the Greek and Dominican Republic material are illustrated by scanning electron micrographs which match the illustrations of the two species given by Petrovitz (1961). It is concluded that the synonymy of the two species, proposed by Stebnicka & Howden (1995) is erroneous.

INTRODUCTION

In a recent issue of this journal, Krell & Simon (2003) reported on the attraction of *Aphodius pseudolividus* to commercial insect repellent, and added a taxonomic note that the synonymy of *A. pseudolividus* Balthasar, 1941 with *A. lividus* Olivier, 1789, proposed by Stebnicka & Howden (1995) was unjustified and should be rejected. The evidence presented in this paper vindicates that view.

Aphodius lividus was originally described from the environs of Paris and during the 19th century it was apparently widespread in Europe, including southern England (Baraud, 1992; Jessop, 1986), but its range has become more restricted to southern Europe and Africa. Prior to Balthasar's (1941) description of *A. pseudolividus, A. lividus* was listed from most of the warmer regions of the world, including the Caribbean (Chapin, 1940), and this view has persisted. Thus Chalumeau & Gruner (1974) illustrated the aedeagophore of Caribbean "*A. lividus*" which is very clearly *A. pseudolividus*. Petrovitz (1961) reviewed the species of subgenus *Labarrus*, with descriptions of new species and lists of localities from where he had seen material of the various species. These lists indicate that nearly all the species occur in the Ethiopian and Oriental Regions, and strongly suggest that species with broad or even cosmopolitan distributions (like *A. pseudolividus*) have been spread by human activity. Petrovitz illustrated the aedeagophores of all the species.

It is against this background that Stebnicka & Howden (1995), in their revision of the Australian species, placed *A. pseudolividus* as a synonym of *A. lividus* on the grounds that it was "highly polymorphic". In fact it is *A. pseudolividus* which occurs in Australia, just as it is this species which occurs in the Caribbean.

A fortnight's visit to the Dominican Republic by RBA and CJW as part of a research project on the local river fauna, in August 2000, provided an opportunity to collect Scarabaeid material, including *A. pseudolividus*, for chromosomal investigation as part of CJW's Ph.D. research. Karyotypes obtained from this material (Wilson, 2002, included in this paper) showed that several chromosomes had heterochromatic blocks occupying the whole of their long arms, raising the possibility that, as with *A. fimetarius* (L.) and *A. pedellus* (DeGeer) (Wilson, 2001), study of the chromosomes might help delimit the various species of this group. Collection of living *A. lividus* in Greece by DJM in June 2003, gave RBA the opportunity of obtaining its karyotype and comparing it with that of *A. pseudolividus*, as well as preparing scanning electron micrograph (SEM) pictures of the aedeagophores of the two species. DJM's South African material of *A. pseudolividus*, collected in September 2003, confirmed the karyotype obtained from Dominican material, but also revealed some variation in the X chromosome, while a single *A. lividus* collected on Cyprus by RBA in April 2004 has a karyotype matching the Greek material.

MATERIAL AND METHODS

Details of the beetles used in the present study are given in Table 1.

Chromosome preparations were from mid-gut and testis, using the methods described by Shaarawi & Angus (1991). The times in colchicine and hypotonic potassium chloride were $12^1/_2$ min. C-banding, which picks out regions of the chromosome where the DNA is highly repetitive (the same base-pair or short sequence of base-pairs repeated many times – constitutive heterochromatin) was done using a saturated solution of barium hydroxide for 5 min. at room temperature (ca 23°C), followed by 1 hour in salt-sodium citrate (2 × SSC) at 60°C. Photographs were taken using a precision interference filter to give a monochromatic green light, and Agfa Copex Rapid AHU high-contrast microfilm. Slides were

TABLE 1. — BEETLES USED FOR CHROMOSOME ANALYSIS.

Species	Country	Region	Locality	No. specimens yielding karyotypes
A. lividus	Greece	Poros Is.	Leondopolou	2♂♂
	Cyprus	Pafos district	Cape Drepanon	1♀
A. pseudolividus	Dominican Republic	Samana Espaillat	El Limón	1♂, 1♀
			Las Flores near Sta. Maria	1♀
	South Africa	Cape Province	Kerpsted	1♂, 1♀

photographed under oil immersion, without a coverslip. The immersion oil can be removed with xylene, and the preparations destained either by a short (5 minute) treatment with 2 × SSC at 60°C., or by refixing in 3 : 1 ethanol/acetic acid. The preparations may then be C-banded, often successfully. Karyotypes were prepared by cutting the chromosomes from photographs printed at a magnification of 3000 ×, matching homologous chromosomes and arranging the pairs of autosomes in rows, in order of decreasing size from left to right. The sex chromosomes were placed at the right hand end of the rows, followed by any B-chromosomes. Relative Chromosome Lengths (RCL, the length of each chromosome expressed as a percentage of half the total lengths of the autosomes in the nucleus), were calculated by measuring the chromosomes with a ruler. They were used as a rough guide only, to facilitate comparisons of the two species. For this reason no statistical analyses were undertaken.

Aedeagophores were mounted unplated on electron microscope stubs and scanned using back-scattered electrons. The specimens were card-mounted and are kept in our collections.

RESULTS

Aedeagophores

SEM pictures of the aedeagophores of the two species are shown in fig. 1. The identity of the *A. lividus* is confirmed by the fact that it is the only species of the subgenus *Labarrus* known from Europe, and the aedeagophore matches the figures given by Baraud (1992) and Petrovitz (1961). The material also keys to *A. lividus* in the works of Balthasar (1941) and Petrovitz (1961). The identity of the *A. pseudolividus* (originally described from near Buenos Aires in Argentina) was

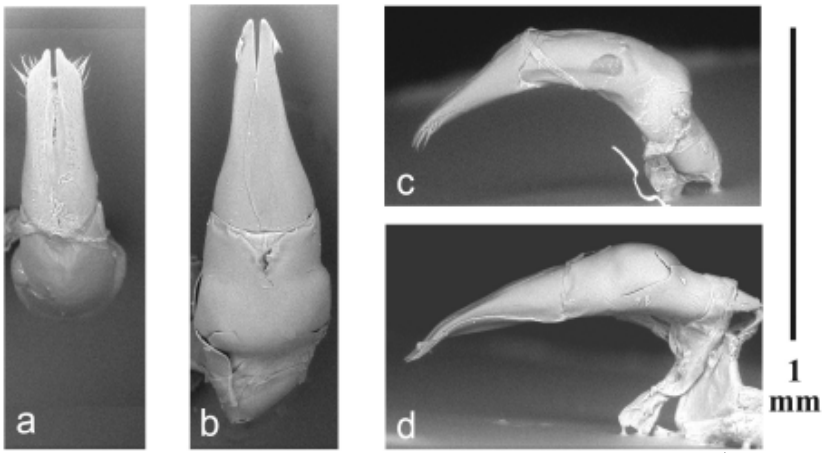


Fig. 1. — a–d. Scanning electron micrographs of *Aphodius* aedeagophores. a,c, *A. lividus* from Poros, Greece; b,d, *A. pseudolividus* from Samana, Dominican Republic. a,b, dorsal view, c,d, lateral view.

confirmed by reference to Balthasar's (l.c.) key. The aedeagophore matches that figured by Petrovitz (1961). Thus the two species studied here are shown to be *A. lividus* and *A. pseudolividus*. The aedeagophore of the South African male is the same as the Dominican one figured.

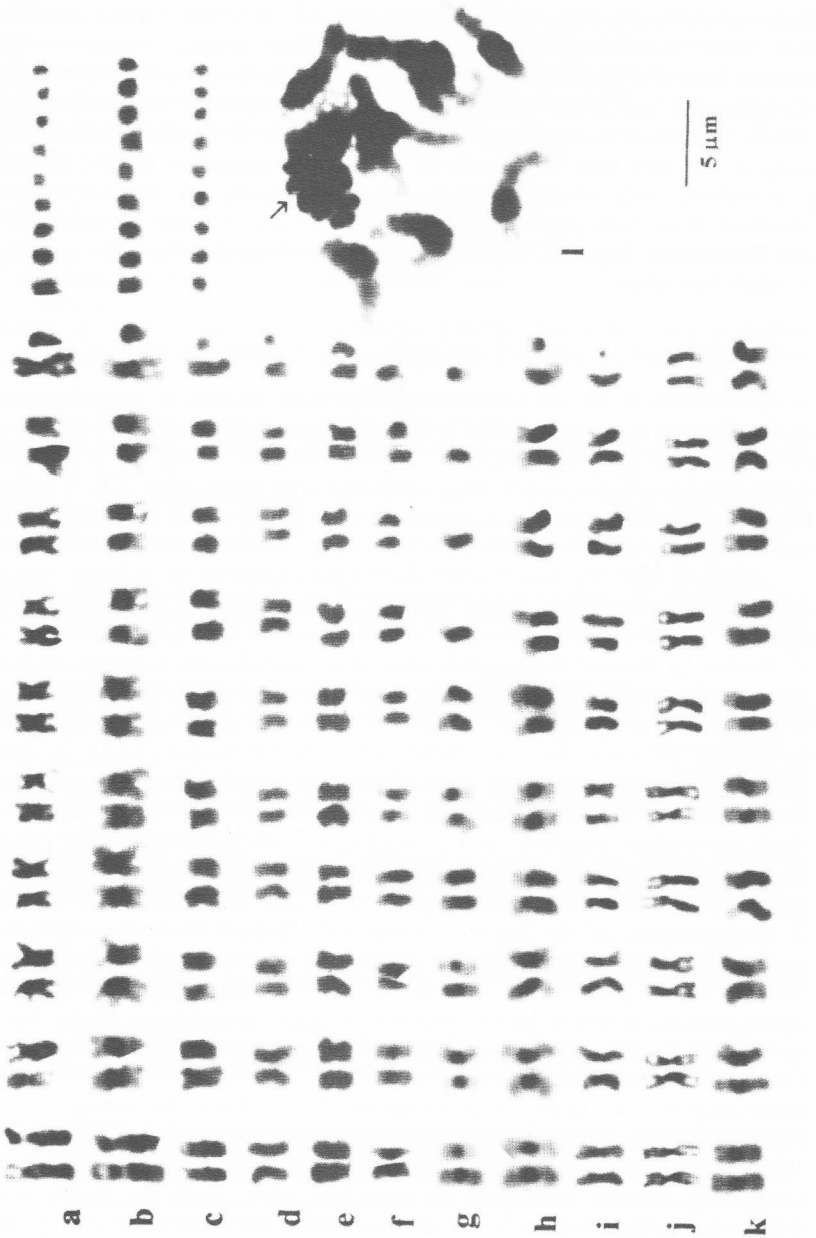
Chromosomes

A. lividus. Fig. 2a–c, l. 2N = 18 + Xy + 9 B-chromosomes (♂, Poros), 18 + XX + 3 B-chromosomes (♀, Cyprus). The RCLs of the autosomes range from about 17–10, with autosome 1 about 17, autosome 2 about 13, autosomes 3–9 about 10, the X chromosome about 17, the y chromosome about 7, and the B-chromosomes from about 7 to about 4. Autosomes 1 and 7 are submetacentric, with the shorter arm about half the length of the longer one. Autosomes 2–6 and the X chromosome are metacentric, with the two arms more or less the same length, while autosomes 8 and 9 and the y chromosome are acrocentric, with the centromere almost terminal. The B-chromosomes are metacentric to submetacentric. C-banding (fig. 1b,c) shows autosome 1 to have heterochromatic blocks (C-bands) at the base of the arms, adjacent to the centromere, with the block on the short arm about twice the size of the one on the long arm. There is another, larger block in the middle of the long arm. Autosomes 2–9 and the X chromosome, have heavy C-bands at the centromere. The y chromosome is largely heterochromatic, while the B-chromosomes are either entirely heterochromatic or nearly so. In the first division of meiosis (fig. 1l) the B-chromosomes and the sex chromosomes condense precociously and are clustered together.

A. pseudolividus. Fig. 2d–k. 2N = 18 + Xy. The RCLs of the autosomes range from about 14 to 9, with the X chromosome similar in length to autosome 9, RCL about 9 and the y chromosome dot-like, RCL about 4. All the autosomes are more or less metacentric, and the X chromosome is clearly acrocentric in Dominican material (fig. 2d–g), but with a distinct heterochromatic short arm in South African material (fig. 2h–k). C-banding (fig. 2f–h,k) shows that autosomes 4 and 6–9 each have one arm entirely heterochromatic. Autosomes 1–3 and 5 have fairly small but distinct centromeric C-bands. The centromeric C-band of the X chromosome extends over the basal half of the long arm. In the material from the

(See facing page)

Fig. 2. — a–l, chromosomes of *Aphodius lividus* and *A. pseudolividus*. a–k, karyotypes assembled from mid-gut mitotic metaphases. a, *A. lividus*, ♂, Poros, plain; b, the same nucleus, C-banded; c, *A. lividus*, ♂, Poros, a more condensed preparation for comparison with *A. pseudolividus*; d, *A. pseudolividus*, ♂, Samana, plain; e & f, *A. pseudolividus*, ♀, Las Flores, e plain, f C-banded, 1 X chromosome missing; g, *A. pseudolividus*, ♂, Samana, C-banded, 3 autosomes and the y chromosome missing; h,i, *A. pseudolividus*, ♂, South Africa, h C-banded, i the same nucleus, plain; j,k, *A. pseudolividus*, ♀, South Africa, j plain, k C-banded. l, *A. lividus*, ♂, Poros, prophase of first division of meiosis showing the cluster of B-chromosomes (arrowed) associated with the sex chromosomes, all much more condensed than the autosomes.


1
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6
7
8
9
X^Y/Y
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b
c
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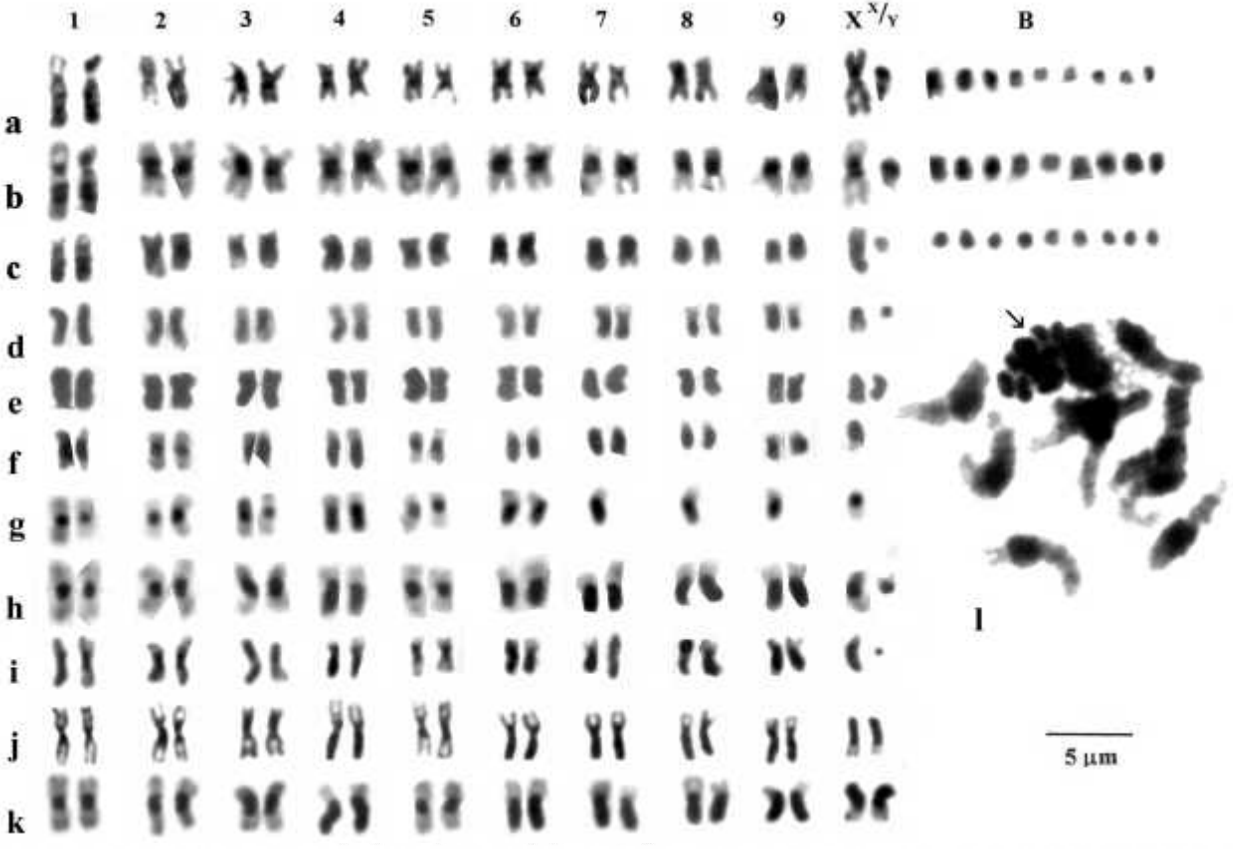
k
I
5 µm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X X/Y
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5 µm

Dominican Republic the short arm of the X chromosome is very small, but in the C-banded partial karyotype shown in fig. 2g there is a small slightly fuzzy euchromatic region distal to the C-band. In South African material this short arm is clearly larger, and may appear either totally heterochromatic (fig. 2k) or only partly so, darker than the long arm of the chromosome but clearly paler than the main centromeric C-band (fig. 2i). These features (fuzziness, variable response to C-banding treatments) suggest that this may be the site of a nucleolus organiser (NOR) in both populations, and, along with the close similarity of the karyotypes obtained from both populations, support the view that the material from the Dominican Republic and South Africa belongs to the same species, *A. pseudolividus*.

CONCLUSIONS

The extent of the chromosomal differences shown to exist between *A. lividus* and *A. pseudolividus* is far too great for the two to be regarded as conspecific. For example, in *A. lividus* autosome 1 and the X chromosome appear conspicuously long compared with autosomes 2–9, whereas this is not at all the case in *A. pseudolividus*, where the X chromosome is one of the smaller ones. Even if the long heterochromatic blocks in *A. pseudolividus* are discounted (heterochromatin is to a large extent genetically inert), there would have to be considerable translocation of material between chromosomes to get from the arrangement found in *A. lividus* to that in *A. pseudolividus*. Such translocation would disrupt pairing of homologous chromosomes of hybrids during meiosis, leading to sterility. The integrity of *A. pseudolividus* is supported by the broad agreement between the karyotypes of Dominican and South African *A. pseudolividus* – especially clear when the C-banded karyotypes shown in fig. 2g and f are compared. Not only do the sequences of chromosome sizes, centromere positions and C-band sizes match, but darkening of the shorter arms of autosome 2, suggesting the presence of a nucleolus organiser, is the same in both karyotypes. The difference between the X chromosomes of the South African and Dominican *A. pseudolividus* appears to be associated with the possession of a nucleolus organiser at this site in both populations and may be taken as illustrating the degree of chromosomal variation found between different populations of the same species. It should also be noted that, if the current view that the present almost cosmopolitan distribution of *A. pseudolividus* is largely anthropogenic is correct, the localities from which the material was obtained do not reflect genuine geographic variation within the species. The SEM illustrations of the aedeagophores of the two species given here agree with relevant published pictures, and, along with the clarity with which the material keyed out in the works of Balthasar (1941) and Petrovitz (1961) vindicate the treatment of the species by these authors.

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