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A karyosystematic investigation of a group of sibling species related to *Stictotarsus griseostriatus* (De Geer) (Coleoptera: Dytiscidae)

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Abstract. A karyological investigation has been carried out on material of the *Stictotarsus griseostriatus*-group of species from 19 localities in Europe and Asia Minor. Seven karyotypically distinct species have been recognised: *S. griseostriatus* De Geer from the Baltic coast of Sweden, *S. multilineatus* Falkenström from inland Sweden and Scotland, and five new species: *S. alpestris* **sp. n.** from the central and eastern Alps, *S. inexpectatus* **sp. n.** from the western Alps, *S. ibericus* **sp. n.** from the Iberian Peninsula and western Alps, *S. creticus* **sp. n.** from Crete and *S. riberae* **sp. n.** from Bulgaria and Turkey. Chromosome numbers range from 2n = 60 + X0 (♂), XX (♀) (*S. griseostriatus*) to 2n = 52 + X0 (♂), XX (♀) (*S. ibericus*, *S. creticus*). In addition, *S. ibericus* shows a fusion-fission polymorphism between two pairs of autosomes. It is considered that the differences between the karyotypes are such that any hybrids would be sterile. Aedeagi and parameres are illustrated, but it is considered that the morphological distinctions between the species are virtually non-existent. The distributions of the species are discussed and the complexities arising from range-changes resulting from Pleistocene climatic fluctuations are noted.

Key words: *Stictotarsus griseostriatus*, Dytiscidae, chromosomes, karyotypes, sibling species.

INTRODUCTION

Stictotarsus griseostriatus (De Geer), in the broadest sense, is a small Dytiscid water beetle widely distributed in small water bodies on the treeless regions of the northern Holarctic, and in isolated high-altitude pools further south. Adults are capable fliers, and their distribution extends to offshore islands such as the British Outer Hebrides (Jackson, 1956).

The beetles show noticeable, though slight, morphological variation, with some specimens paler and having the black markings well separated from one another, while others are darker with the black markings tending to be confluent. There are also minor differences in body shape. This variation has led to a number of different species being described, most notably the separation by Falkenström (1922, 1930) of *S. multilineatus* (from alpine districts of Sweden) from *S. griseostriatus* (from the Baltic coast). This fragmentation of *S. griseostriatus* has been rejected by the majority of mainstream specialists on the group. Thus Zaitzev (1953) noted that if adequate samples from any population were studied, all the variation in colour pattern and pronotal shape which has been used to separate different species would be found to be present. The first indication that this monolithic view of *S. griseostriatus* was untenable came from chromosomal studies by R.B. Angus (mentioned in Nilsson, Angus, 1992). In view of these results *S. griseostriatus* and *S. multilineatus* were treated as separate species by A.N. Nilsson and M. Holmen (Nilsson, Holmen, 1995).

The present study reports investigation of the karyotypes of material from 19 populations from various parts of Europe. It results in the recognition of seven distinct species, five of which are described as new.

Holotypes of new species are placed in the Natural History Museum, London. Unless otherwise stated, paratypes are in R. B. Angus' collection.

Material and methods

The material studied is listed in Table 1, under the names of the species here recognised. A map showing the sampling localities is given as Fig. 1.

Living beetles were kept in aquaria at room temperature, and fed with living *Tubifex* worms (Oligochaeta). Chromosome preparations were made from mid-gut, ovary and testis, using the methods described by F.A. Shaarawi and R.B. Angus (Shaarawi, Angus, 1991). C-banding was carried out on 2-day old slides. Treatment time in Barium hydroxide varied according to room temperature, but 2 minutes at about 27° C usually gave good results. Weakly-staining preparations were sometimes seen more clearly under phase contrast. Preparations were photographed on to

Table 1. Material used for chromosome analysis.

Species	Locality	Collector
Stictotarsus griseostriatus De Geer	Sweden, Upland, Öregrund	B. Svensson
	Sweden,Västerbotten, Råtan	A.N. Nilsson
	Sweden, Ångermanland, Järnäs	A.N. Nilsson
S. multilineatus Falkenström	Sweden, Västerbotten, Åmsele	A.N. Nilsson
	Scotland, Kirkcudbright, Clatteringshaws Loch	G.N. Foster
S. alpestris **sp. n.**	Italy, Dolomites, Falcade	F. Pederzani
	Switzerland, Ticino, Medeglia	A. Focarile
S. inexpectatus **sp. n.**	France, Hautes Alpes, Lac du Lauzet inférieur	M. Drotz
S. ibericus **sp. n.**	Spain, Provincia de Madrid, Peña Lara	R.B. Angus, D.T. Bilton, M. D. Eyre
	Spain, Provincia de León, Puerto de las Señales	L.A. Dutton, R.B. Angus
	Spain, Provincia de Granada, Sierra Nevada	M. Drotz
	Portugal, Serra do Estrela	D.T. Bilton
	Italy, Maritime Alps, Piemonte, Col de la Lombarde	H. Fery
	France, Hautes Alpes, Lac des Pelouses; Lac du Lauzet supérieur; Lac de Guillestre	M. Drotz
S. creticus **sp. n.**	Greece, Crete, Nomos Khanion, Omalos	R.B. and H.J. Angus
S. riberae **sp. n.**	Bulgaria, Pirin mts., Dobriniste	D.T. Bilton
	Turkey, Düzce, Çaydurt-Kartalkaya rd.	I. Ribera

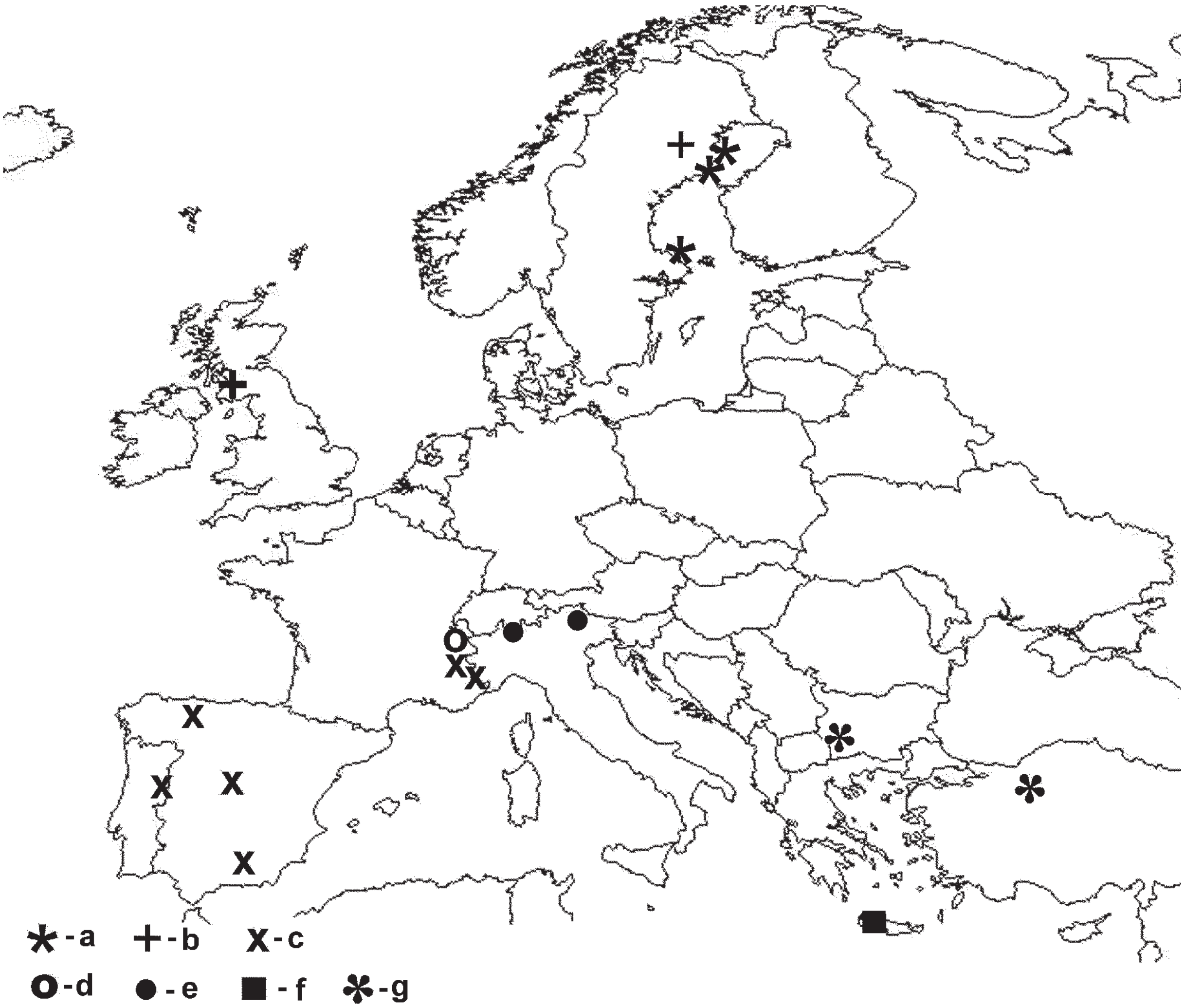

Fig. 1, a-g. Map showing the localities from which material has been collected. **a** - *Stictotarsus griseostriatus*. **b** - *S. multilineatus*. **c** - *S. ibericus* **sp. n.** **d** - *S. inexpectatus* **sp. n.** **e** - *S. alpestris* **sp. n.** **f** - *S. creticus* **sp. n.** **g** - *S. riberae* **sp. n.**

microfilm, printed, and the photographs cut up to assemble karyotypes. These were then scanned into a computer and further arranged using Adobe Photoshop. Specimens from which chromosome preparations were obtained were card-mounted and are kept in R. B. Angus' collection unless otherwise stated.

RESULTS

Representative karyotypes are shown in Fig. 2, and meiotic first metaphases in Fig. 3. Scanning electron micrographs of aedeagophores are shown in Fig. 4 and photographs of parameres in Fig. 5. The species are considered in turn, and because all but two are new, the descriptions are given as the species are discussed.

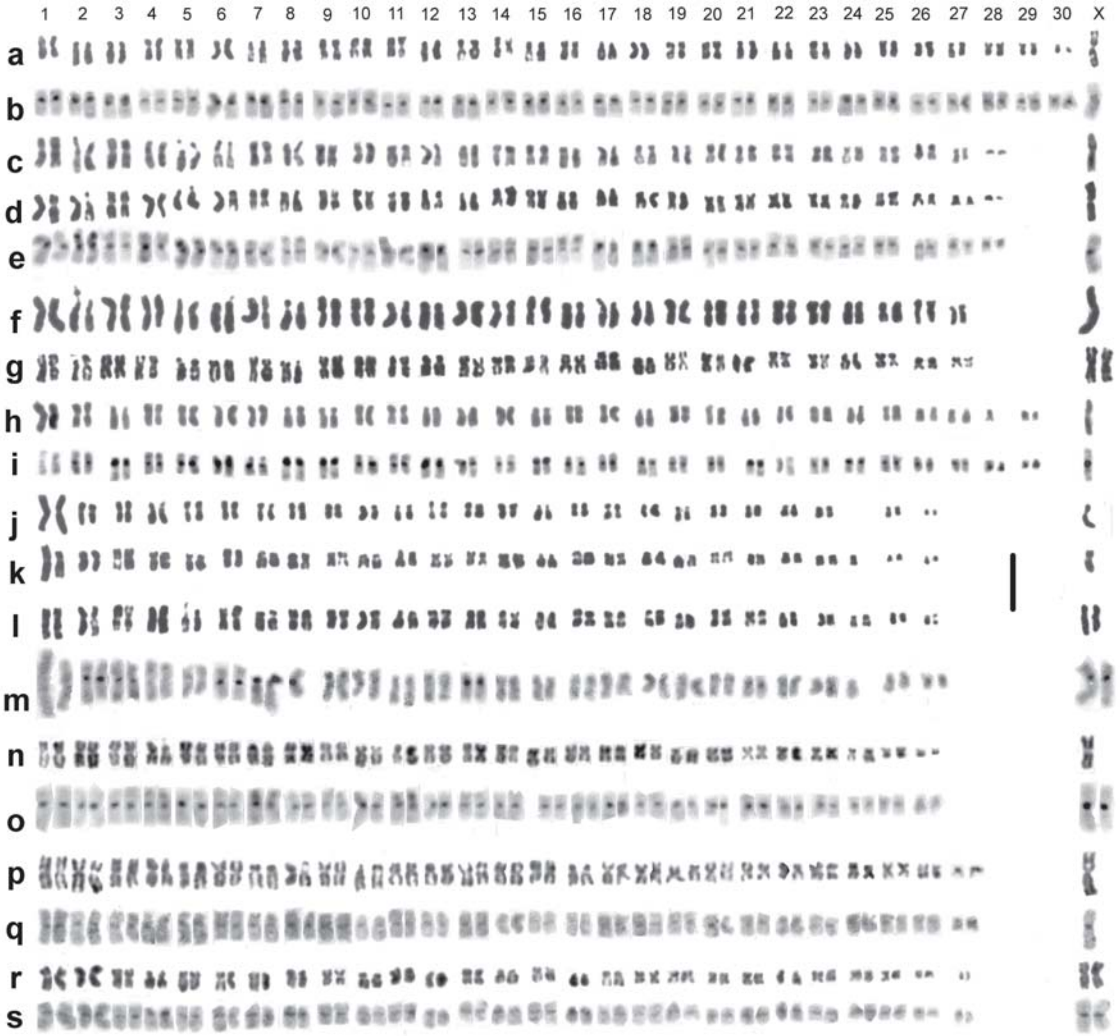

Fig. 2, a-s. Mitotic chromosomes from mid-guts of *Stictotarsus griseostriatus*-group of species, arranged as karyotypes. **a**, **b** - *S. griseostriatus*, ♂♂; **a** - Öregrund, plain; **b** - Järnäs, C-banded. **c-e** - *S. multilineatus*, ♂♂; **c** - Åmsele, plain; **d** - Clatteringshaws, plain; **e** - Clatteringshaws, C-banded. **f, g** - *S. alpestris* **sp. n.**; **f** - ♂, Falcade, paratype, plain; **g** - ♀, Medeglia, paratype, plain. **h**, **i** - *S. inexpectatus* **sp. n.**, ♂♂; **h** - Lauzet inférieur, paratype, plain, 1 replicate of autosome 28 missing; **i** - Lauzet inférieur, holotype, C-banded. **j-m**, *S. ibericus* **sp. n.**; **j** - ♂, Peña Lara, paratype, plain, autosomes 1 and 24 homozygous fused; **k** - ♂, Peña Lara, paratype, plain, autosomes 1 and 24 heterozygous; **l** - ♀, Peña Lara, paratype, plain, autosomes 1 and 24 homozygous unfused; **m** - ♀, Lauzet supérieur, paratype, C-banded, autosomes 1 and 24 heterozygous. **n**, **o** - *S. creticus* **sp. n.**; **n** - ♂, Omalos, holotype, plain; **o**-♀, paratype, C-banded. **p-s** - *S. riberae* **sp. n.**; **p** - ♂, Turkey, paratype, plain; **q** - the same nucleus, C-banded; **r** - ♀, Bulgaria, holotype, plain; **s** - the same nucleus, C-banded. Bar = 5 µm.

S. griseostriatus De Geer, 1774

Chromosome data. 2n = 60 + X0 (♂), XX (♀). Plain and C-banded karyotypes are shown in Fig. 2, a, b. With 30 pairs of autosomes this is the largest karyotype encountered. All the chromosomes show distinct centromeric C-bands,

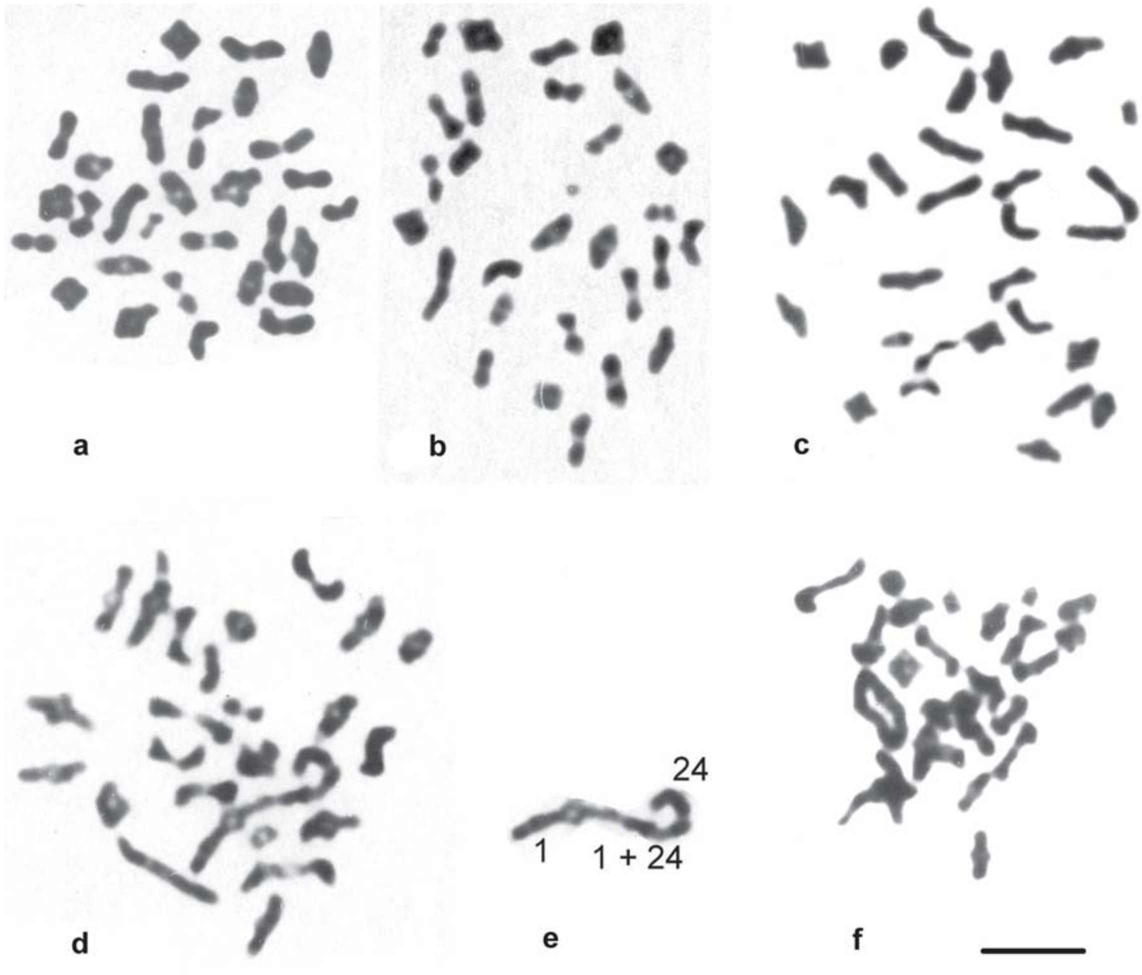

Fig. 3, a-f. Meiosis, first metaphase from testis of *Stictotarsus griseostriatus*-group of species. **a -** *S. griseostriatus*, Öregrund. **b -** *S. multilineatus*, Clatteringshaws. **c -** *S. inexpectatus* **sp. n.**, Lauzet inférieur, paratype. **d-f -** *S. ibericus* **sp. n.**, Peña Lara, paratype; **d -** autosomes 1 and 24 heterozygous for fusion; **e -** trivalent autosomes 1 and 24 from Fig. 3, d, labelled; **f -** autosomes 1 and 24 homozygous fused, forming a large ring-bivalent. Bar = 5 µm.

and five pairs of autosomes (pairs 2, 3, 7, 22 and 24 appear to show secondary constrictions on their short arms, adjacent to the centromeres. The metacentric X chromosome is the largest in the nucleus. The karyotypes of material from the three Swedish localities (Table 1) show no differences from one another. First metaphase of meiosis is shown in Fig. 3, a. 31 elements are present, 30 bivalents and the unpaired X chromosome. The unpaired X chromosome cannot be identified in this unbanded preparation.

Morphology. The aedeagus is shown in Fig. 4, a-c, and the paramere is Fig. 5, a.

Distribution. *S. griseostriatus* appears to be confined to coastal rocky areas of Fennoscandia and the adjacent parts of Russia, including Lake

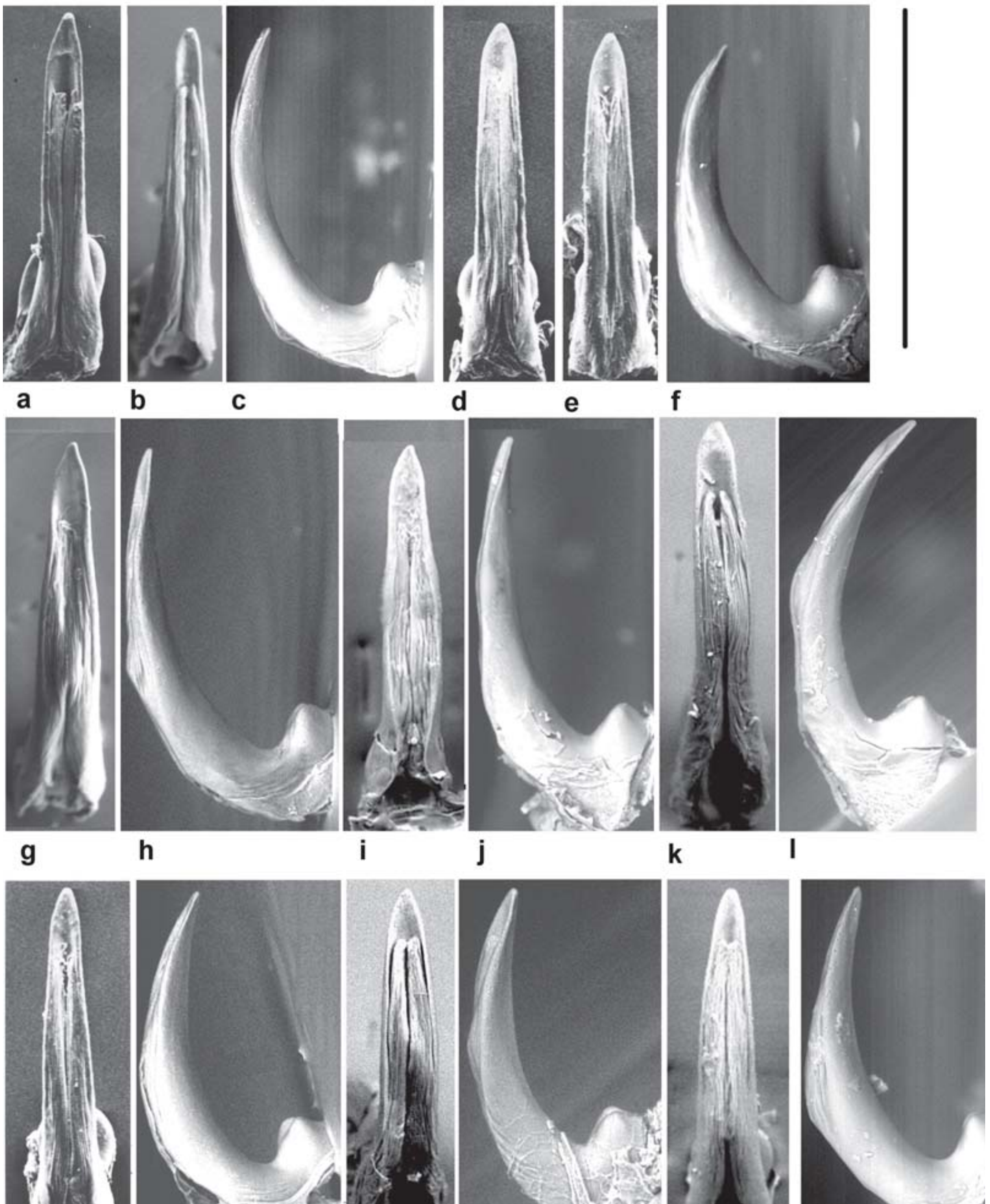

Fig. 4, a-r. Scanning electron micrographs of aedeagi of *Stictotarsus griseostriatus*-group of species. **a**-**c** - *S. griseostriatus*; **a** - Öregrund, dorsal; **b** - Järnäs, dorsal; **c** - Järnäs, lateral. **d-f** - *S. multilineatus*; **d** - Åmsele, dorsal; **e** - Clatteringshaws, dorsal; **f** - Clatteringshaws, lateral. **g-j** - *S. alpestris* **sp. n.**; **g** - Falcade, holotype, dorsal; **h** - Falcade, holotype, lateral; **i** - Medeglia, paratype, dorsal; **j** - Medeglia, paratype lateral. **k**, **l** - *S. inexpectatus* **sp. n.**, Lauzet inférieur, paratype; **k** - dorsal; **l** - lateral. **m**, **n** - *S. ibericus* **sp. n.**, Peña Lara, paratype; **m** - dorsal; **n** - lateral. **o**, **p** - *S. creticus* **sp. n.**, Omalos, paratype; **o** - dorsal; **p** - lateral. **q**, **r** - *S. riberae* **sp. n.**, Turkey, paratype; **q** - dorsal, **r** - lateral. Bar = 0.5 mm.

Ladoga (Lindberg, 1930; Brinck, 1943).

S. multilineatus Falkenström, 1922

Chromosome data. 2n = 56 + X0 (♂), XX (♀). A plain karyotype from Swedish material is shown in Fig. 2, c and plain and C-banded karyotypes from Scottish material are shown in Fig. 2, d, e. The large metacentric X chromosome seems very similar to that of *S. griseostriatus*, but the autosomes are not sufficiently distinctive to suggest homologies between individual chromosomes of *S. multilineatus* and *S. griseostriatus*. All the chromosomes have distinct centromeric C-bands. Autosome pairs 2, 5, 12, 17 and 18 are acrocentric or subacrocentric and with distinct secondary constrictions on their short arms, pairs 6 and 11 are similar but the secondary constrictions are less clear, and pair 26 is simply acrocentric. The remainder are metacentric, and pair 28 is very small. First metaphase of meiosis is shown in Fig. 3, b, with 29 elements (28 bivalents +X) present. As with *S. griseostriatus*, the unpaired X chromosome cannot be identified.

Morphology. The aedeagus is shown in Fig. 4, d-f, and parameres in Fig. 5, b, c.

Distribution. *S. multilineatus* is widely distributed over upland areas of Fennoscandia and northern Britain. It may be more widely distributed but chromosome data would be needed to verify this.

S. alpestris sp. n.

Chromosome data. 2n = 54+X0 (♂), XX (♀). Plain karyotypes, male and female, are shown in Fig. 2, f, g. No C-banded material is available. The sizes and shapes of all the chromosomes, and the sequence of metacentrics, subacrocentrics and acrocentrics, appear to match those of *S. multilineatus* exactly, with only the smallest autosome pair of *S. multilineatus* being absent. Such secondary constrictions as can be recognised also appear to correspond with counterparts in *S. multilineatus*. No preparation of meiosis is variable.

Morphology. The aedeagus is shown in Fig. 4, g-j. It appears to be consistently slightly longer than that of *S. multilineatus*, length about 0.56 mm as against about 0.52 mm. This is an 8 % size difference. Parameres are shown in Fig. 5, d, e. They appear slightly larger and more elongate than those of *S. multilineatus* (Fig. 5, b, c), but their appearance is somewhat distorted because the material is very soft (teneral). Body length: 4.2-4.5 mm (Dolomites), 4.0-4.2 mm (Ticino). The Dolomites material is strongly marked with bold black markings, the elytral lines tending to be confluent. In contrast, the Ticino material has the dark markings pale to mid brown, and the legs and antennae pale. In one specimen the elytral lines are very indistinct.

Holotype. ♂, Italy, Dolomite Alps, Falcade, Col Margherita, Laste di Pradazzo, lake, 2335 m. 18.IX.1993. F. Pederzani. Chromosome preparation 6, 27.IX.1993, R.B. Angus.

Paratypes. 3 ♂♂, 5 ♀♀, provenance as holotype. Chromosome preparations: 1 and 3, 27.IX.1993, 1, 29.IX.1993, R. B. Angus (♂♂); 2, 4, 5, 27.IX.1993, 2 and 3, 29.IX.1993, R.B. Angus (♀♀). 3 ♂♂, 2 ♀♀, Switzerland, Ticino, Above Medeglia, VIII.1994, A. Focarile. Chromosome preparations: 1, 12. VIII.1994, 2 and 4, 18.VIII.1994, R. B. Angus (♂♂); 1 and 3, 18.VIII.1994, R.B. Angus (♀♀).

Distribution. At present *S. alpestris* is known only from the central and eastern Alps.

S. inexpectatus sp. n.

Chromosome data. 2n = 58 + X0 (♂), XX (♀). Plain and C-banded male karyotypes are shown in Fig. 2, h, i. Two pairs of autosomes (Nos 1 and 22) lack C-bands, but all the others, and the X chromosome, have distinct centromeric C-bands. Eight pairs of autosomes are more or less acrocentric, two are submetacentric with distinct

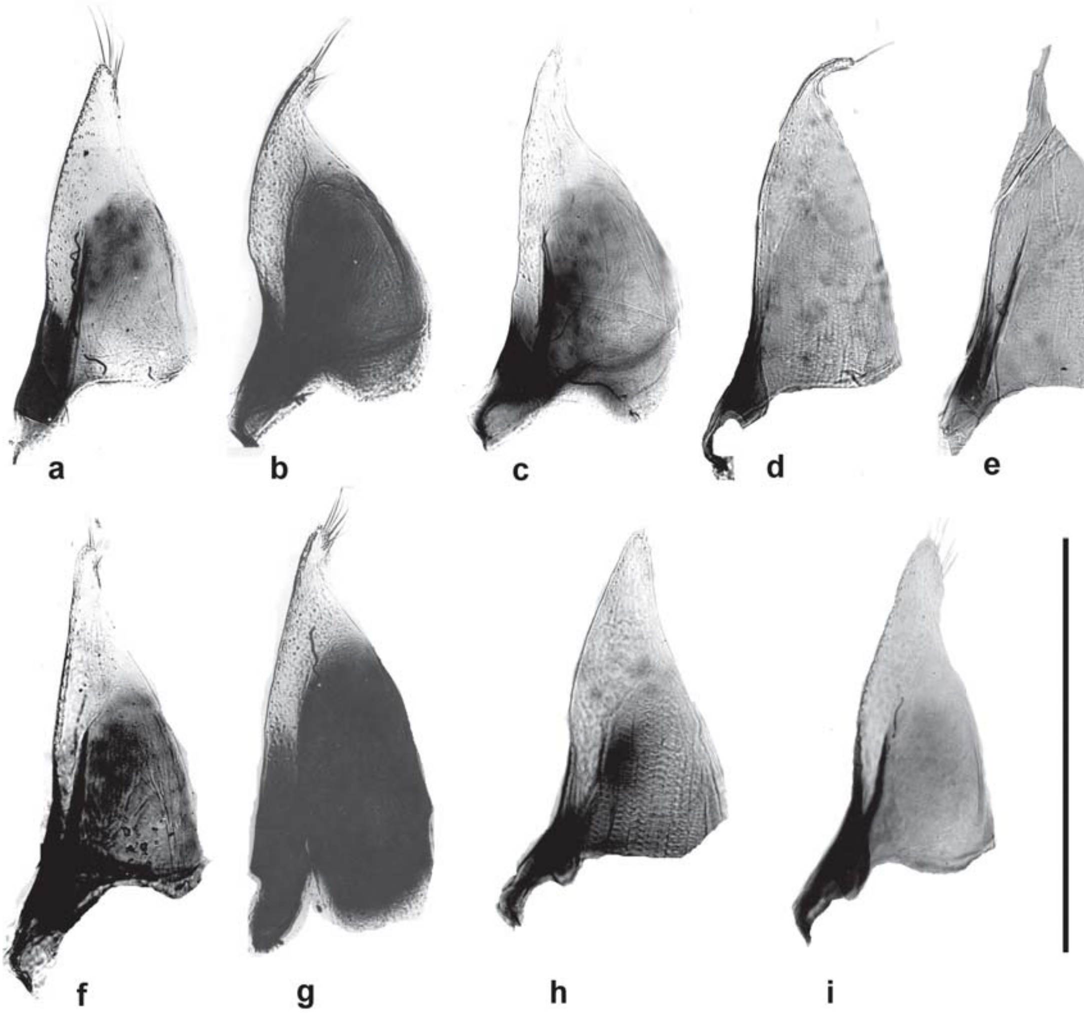

Fig. 5, a-i. Photomicrographs of parameres of *Stictotarsus griseostriatus*-group of species. **a -** *S. griseostriatus*, Öregrund. **b -** *S. multilineatus*, Åmsele. **c -** *S. multilineatus*, Clatteringshaws. **d**, **e -** *S. alpestris* **sp. n.**; **d -** Falcade, holotype; **e -** Medeglia, paratype. **f -** *S. inexpectatus* **sp. n.**, Lauzet inférieur, paratype. **g -** *S. ibericus* **sp. n.**, Peña Lara, paratype. **h -** *S. creticus* **sp. n.**, Omalos, paratype. **i -** *S. riberae* **sp. n.**, Turkey, paratype. Bar = 0.5 mm.

secondary constrictions on the short arm, and the remainder are either metacentric or submetacentric.

The metacentric X chromosome is the largest in the nucleus. First metaphase of meiosis, showing 30 elements (29 bivalents plus the X chromosome), is shown in Fig. 3, c. As in *S. griseostriatus* and *S. multilineatus*, it is not possible to recognise the unpaired X chromosome. This karyotype, second in length only to that of *S. griseostriatus*, is unlike that of any other western or central European species. The species with distributions adjacent to it (*S. alpestris* and *S. ibericus*) have considerably shorter karyotypes (2n = 54 + X0, XX and 2n = 52 + X0, XX).

Morphology. The aedeagus is shown in Fig. 4, k, l. It is about 0.59 mm long, about 6 % longer than that of *S. alpestris*. The tip is noticeably blunt. The parameres (Fig. 5, f) are distinctly longer and more elongate than those of *S. multilineatus* (Fig. 5, b, c), and slightly more elongate than those of *S. ibericus* (Fig. 5, g). Body length: 4.2-4.5 mm. The beetles are boldly marked with the black markings discrete and very distinct from the yellowish background. The specimens available appear rather elongate, with the lateral margins less rounded than in some specimens of the complex. However, as this species is so far known from only one lake it is not possible to consider the extent of its morphological variation.

Holotype. ♂, France, Hautes Alpes, Briançon St Crépin, Lac du Lauzet inférieur, 2450 m, 44°44’45.9”N/06°40’8.58”E, 28.VIII.1998, M. Drotz. Chromosome preparation 2, 30.XI.1998, R.B. Angus.

Paratypes. 4 ♂♂, 2 ♀♀, provenance as holotype. Chromosome preparations: 1-3, 13.XI.1998, 4, 30.XI.1998, R.B. Angus (♂♂); 1 and 3, 30.XI.1998, R.B. Angus (♀♀).

Distribution. Known only from the type locality.

S. ibericus sp. n.

Chromosome data. 2n = 52 + X0 (♂), XX (♀). Plain karyotypes of Spanish material (males and females) are shown in Fig. 2, j-l. A C-banded karyotype from a French female is shown in Fig. 2, m. This is the most distinctive karyotype encountered in the present study. To begin with, it shows a fusion-fission polymorphism involving autosome pairs 1 and 24. Thus the observed chromosome numbers range from 50 – 52 in addition to the X chromosome. Fig. 2, j-l shows specimens homozygous for the fusion, heterozygous, and homozygous for non-fusion. The fused autosome 1 + 24 is by far the longest in the nucleus, and the X chromosome is smaller than in the other species encountered, approximately equal in length to unfused autosome 1. The C-banding pattern is also very different from that shown by the other species, with strong centromeric C-bands showing on only six pairs of autosomes plus the X chromosome. In the remaining autosomes centromeric C-bands are either absent or very weak. The karyotype, including the fusion-fission polymorphism, is similar in all the studied populations (Fig. 1). First metaphase of meiosis is shown in Fig. 3, d, f. Fig. 3, d shows a specimen heterozygous for the fusion-fission polymorphism, with a long trivalent involving autosomes 1, 1+24 and 24. This trivalent is shown separately, with the components labelled, in Fig. 3, e. Fig. 3, f shows a specimen homozygous for the fusion, with autosome 1+24 forming a ring-bivalent.

Morphology. The aedeagus is shown in Fig. 4, m, n. The length, about 0.52 mm, is similar to that of *S. multilineatus*. The parameres (Fig. 5, g) are fairly elongate. Body length: 3.8-4.7 mm. The dark pattern is very distinct in most specimens, with the elytral lines largely not confluent. The body outline is variable, but generally more rounded laterally than *S. inexpectatus*.

Holotype. ♂, Spain, Provincia de Madrid, Sierra Guadarrama, Peña Lara, Laguna de la Hoya de Peña Lara, 1960 m, 11.V.1990, R.B. Angus, D.T. Bilton, M.D. Eyre. Chromosome preparation 1, 20.VIII.1990, R.B. Angus.

Paratypes. 3 ♂♂, 5 ♀♀, provenance data as holotype. Chromosome preparations: 3, 6.VIII.1990; 4, 20.VIII.1990, 1, 31.VII.1990, R.B. Angus (♂♂); 2, 31.VII.1990; 4, 5, 6, 6.VIII.1990; 2 and 3, 20.VIII.1990, R. B. Angus (♀♀); 21 ♀♀, locality data as holotype, but 20.IV.1984, R. B. Angus, G. N. Foster. 34 ♂♂, 20 ♀♀, Spain, Provincia de Madrid, Sierra Guadarrama, Peña Lara, Laguna Grande, 2202 m, 14.IV.1983, R.B. Angus. 3 ♂♂, Spain, Sierra Nevada loc. 3.VIII.1999, M. Drotz. Chromosome preparations: 1-3, 25.IX.1999, R.B. Angus. 2 ♂♂, Spain, Sierra Nevada loc. 12, VIII.1999, M. Drotz. Chromosome preparations: 1, 2, 29.IX.1999, R.B. Angus. 1 ♂, Spain, León, Puerto de las Señales, 1625 m, 20.IV.2006, L.A. Dutton, R.B. Angus. Chromosome preparation 4, 24.IV.2006, L. A. Dutton, R. B. Angus. 3 ♂♂, Portugal, Serra Estrela, pools below Torre, ca 1600 m, V.2005, D.T. Bilton. Chromosome preparations: 1 and 2, 20.V.2005, 3, 8.VI.2005, L.A. Dutton, R.B. Angus. 8 ♂♂, 10♀♀ Italy, Piemonte, Col de la Lombarde 20 km S Vinadio, pool, 2300 m, 15.VII.1994, H. Fery. Chromosome preparations: 1 and 2, 20.VII.1994, 1 and 2, 21.VII.1994, 3, 4.VIII.1994, 2, 25.VIII.1994, 4, 26.VIII.1994, R.B. Angus (♂♂); 3, 20.VII.1994, 3, 21.VII.1994, 1, 26.VII.1994, 1 and 2, 4.VIII.1994, 1 and 3, 25.VIII.1994, R.B. Angus (♀♀). 1 ♂, France, Hautes Alpes, Guillestre, Col de Vars, small pool, 2080 m, 44°32'51.7"N/06°42'24.8"E, 23. VIII.1998, M. Drotz. Chromosome preparation 1, 17.XI.1998, R.B. Angus. 4 ♂♂, 2 ♀♀, France, Hautes Alpes, Jausiers, Lac de Pelouses, 2500 m, 44°21'04.9"N/06°51'57.2"E, 24.VII.1998, M. Drotz. Chromosome preparations: 3, 17.XI.1998, 1-3, 9.XII.1998, R.B. Angus (♂♂), 2, 17.XI.1998, 4, 9.XII.1998, R.B. Angus (♀♀). 6 ♂♂, 4 ♀♀, France, Hautes Alpes, Briançon St Crépin, Lac du Lauzet supérieur, 2250 m, 44°44'49.9"N/06° 40'6.45"E, 28.VIII.1998, M. Drotz. Chromosome preparations: 2 and 3, 16.XI.1998, 5-8, 9.XII.1998, R.B. Angus (♂♂); 5-8, 9.XII.1998, R. B. Angus (♀).

Distribution. *S. ibericus* is known from all the main mountain ranges of the Iberian Peninsula, as well as from the western Alps to the district round Briançon. It is highly likely that Pyrenean material belongs to this species, but we have not seen any chromosomes from there. It is possible that this is the *S. griseostriatus*-group species occurring in the Atlas Mountains of Morocco.

S. creticus sp. n.

Chromosome data. 2n = 52 + X0 (♂), XX (♀). Karyotypes are shown in Fig. 2, n (male, plain) and o (female, C-banded). Although the chromosome number is the same as that of *S. ibericus* (unfused), the karyotypes are clearly different. The X chromosome is the longest in the nucleus and all the chromosomes have distinct centromeric C-bands. Only one pair of autosomes (No. 19) is clearly more or less acrocentric, and pairs 4, 7, 10 and 11 are submetacentric with secondary constrictions in their short arms.

Morphology. The aedeagus is shown in Fig. 4, o, p. It is small, very similar to that of *S. ibericus* (Fig. 4, m, n). The parameres (Fig. 5, h) are small and fairly broad, similar to those of *S. multilineatus* (Fig. 5, b, c), but slightly broader apically. Body length 4.2-4.7 mm. The pattern is very distinct, and the beetles are elongate but distinctly rounded laterally. The pronotal sides vary from being almost straight to distinctly rounded.

Holotype. ♂, Crete, Nomos Khanion, Omalos plateau, crowfoot (*Ranunculus aquatilis* s. l.) pool, 1100m, 9.IV.1996, R.B. Angus. Chromosome preparation 1, 19.IV.1996, R.B. Angus.

Paratypes. 27 ♂♂, 17 ♀♀, provenance as holotype. Chromosome preparations: 1 and 2, 18.IV.1996, 3, 19.V.1996, 4-6, 24.IV.1996, 1-3, 14.VI.1996, 4-6, 1.VII.1996, 2 and 4, 18.VII.1996, 1 and 2, 25.VII.1996 (♂♂); 2, 19.IV.1996, 5 and 6, 1.VII.1996, 1 and 3, 18.VII.1996, 3 and 4, 5.VII.1996 (♀♀). 10 ♂♂ and 10 ♀♀, without chromosome preparations.

Distribution. *S. creticus* is known only from the type locality.

S. riberae sp. n.

Chromosome data. 2n = 54 + X0 (♂), XX (♀). A male karyotype from a Turkish specimen is shown if Fig. 2, p (plain) and q (C-banded). A female karyotype from a Bulgarian specimen is shown in Fig. 2, r (plain) and s (C-banded). All the chromosomes have distinct centromeric C-bands, and the metacentric X chromosome is clearly the longest in the nucleus. At a first glance the chromosomes appear similar to those of *S.*

alpestris (Fig. 2, f, g), but the very small autosome pair 27 does not match. Thus in *S. alpestris* it appears that autosome pair 28 of *S. multilineatus* (Fig. 2, c-e) is missing, but in *S. riberae* it would be pair 27 that is missing, with pair 28 retained. Once karyotypes are assembled, other differences emerge. In particular, the longest autosome with a secondary constriction appears as pair No. 2 in *S. multilineatus* and *S. alpestris*, but as pair No. 4 in *S. riberae*. Even allowing for some uncertainties in the positioning of chromosome pairs in the karyotype, this chromosome has a consistently shorter relative length in *S. riberae* than in the other two species. Other submetacentric or subacrocentric chromosomes with secondary constrictions in their short arms are placed as pairs 4, 5, 8, 12, 16 and 22, while acrocentric or subacrocentric chromosomes without obvious secondary constrictions are placed as pairs 10, 11 and 14. The remaining chromosomes are metacentric or submetacentric.

The karyotypes obtained from Bulgarian and Turkish material match well, and their general arrangements do not match those of *S. multilineatus* and *S. alpestris*. The differences between these karyotypes would require a number of translocations to get from one to the other, which would render any hybrids infertile.

Morphology. The aedeagus (Fig. 4, q, r) is small, not obviously different from those of *S. creticus* and *S. ibericus* (Fig. 4, m-p). The parameres of the single male studied (Fig. 5, i) are noticeably elongate, more like *S. ibericus* (Fig. 5, g) than *S. creticus* (Fig. 5, h). Body length: 4.6-4.9 mm (Bulgarian ♀♀), about 4.6 mm (Turkish ♂). The Turkish ♂ has been used for DNA studies and is in pieces. The Bulgarian material has the black pattern heavily marked, with the elytral lines wider than the pale spaces between them, and confluent in places. The Turkish elytron has narrower dark lines, with the pale spaces wider, and hardly any confluence between the dark lines.

Holotype. ♀, Bulgaria, Pirin mts., alpine rock pools above Dobriniste, 2000 m, 5.VII.2006, D. T. Bilton. Chromosome preparation 1, 14.VII.2006, L.A. Dutton, R.B. Angus.

Paratypes. 4 ♀♀, provenance as holotype. Chromosome preparations 2 and 3, 14.VII.2006, 1, 15.VII.2006, 1, 17.VII.2006, L.A. Dutton, R.B. Angus. 1 ♂, Turkey, Düzce, pools on mountain pass, 1700 m, Kartalkaya-Çaydurt rd. 40°40’20”N/31°47’05”E, I. Ribera. DNA reference No. MNCN-AI-829. Chromosome preparation 7, 8.V.2006, L.A. Dutton, R.B. Angus. In the Museo Nacional de Ciencias Naturales, Madrid.

Etymology. This species is named after Dr Ignacio Ribera (Madrid), with Ribera taken as a first declension masculine noun.

Distribution. At present *S. riberae* is known only from the two localities in Bulgaria and Turkey, but it may very well be more widely distributed in Eastern Europe and Asia Minor.

Discussion

The results presented here reveal a complex of distinct but morphologically very similar species whose distributions, though often adjacent, do not appear to overlap. Some caution is necessary here, however. Some at least of the species are good fliers, and sampling even 10 individuals from a lake would be unlikely to detect a small proportion of immigrants. This type of distribution pattern involving chromosomally distinct species is well known in the Orthoptera (e.g. Shaw et al., 1980), and is often regarded as involving parapatric speciation. However, in this case the present distributions of the species may be misleading. For example, *S. griseostriatus* has a coastal distribution in Scandinavia and Finland, while *S. multilineatus* inhabits the northern hinterland. However, at the height of the Last Glaciation, about 35 000 years ago, all this land was buried under an ice sheet, and as recently as 15 000 years ago the whole of Fennoscandia was still ice-covered (Lowe, Walker, 1997: 271). Therefore these species must have migrated to their present ranges comparatively recently. Coope (1979) gives an account of the changes in the distribution of British beetle species in response to Pleistocene climatic oscillations. The situation in Central and Southern Europe is more complicated and in general less well known. However, the Iberian Peninsula has a rich suite of endemic water beetles, many of them apparently originating in the Pleistocene (Ribera, 2003).

The known ranges of the species vary considerably in size, irrespective of the ages of their present distributions. Thus on the one hand *S. multilineatus*, a recent immigrant to its present range, is widely distributed over Scandinavia and northern Britain, while in contrast the Alpine *S. inexpectatus* and *S. alpestris* have very restricted distributions, though these species may have survived the Pleistocene climatic fluctuations by merely moving up and down the mountains. This pattern may in fact be entirely consistent with the history of the species. *S. multilineatus*, a northern species, will have undergone large range-changes in response to climatic fluctuations, and this will have prevented local populations from being isolated from one another long enough for speciation to occur. If Alpine species were involved in smaller-scale movements, their isolation from one another may not have been so disrupted. Angus (1983) discusses this with respect to the lack of speciation between Spanish and Swedish *Helophorus lapponicus* Thomson, 1854 (Hydrophiloidea).

The distribution of *S. ibericus* is of particular interest. The main Iberian area of its distribution suggests that it is likely to have originated in the Iberian Peninsula, and there survived the Pleistocene. At some stage it appears to have advanced into the western Alps, where its range now abuts that of *S. inexpectatus*.

From a practical point of view, the most difficult aspect of this species complex is that morphologically most of the species are virtually indistinguishable from one another. The karyotype differences found between the species are such that considerable chromosomal translocation would be needed to change from one to another, and it is therefore very difficult to see how hybrids between them could be fertile. However, the fusion-fission polymorphism shown by *S. ibericus* (consistent through its entire range) shows that some chromosomal variation can be accommodated within a species, even though trivalents are formed when heterozygous animals undergo meiosis.

Separation of species of the *S. griseostriatus*-group on the basis of morphology is at best of limited value. The minor variation in the shape and size of the aedeagus shown by some of the species would not be sufficient to lead to mechanical isolation between them, not least because fertilisation in this group of Dytiscidae involves a spermatophore, and this enables successful cross-fertilisation of species with very different aedeagus shapes, such as *Nebrioporus depressus* Fabricius, 1775 and *N. elegans* Panzer, 1794 (Shirt, Angus, 1992).

In the case of *S. griseostriatus* and *S. multilineatus* in Scandinavia and Finland recognition on the basis of morphology appears to be fairly reliable, with *S. griseostriatus* largely confined to rocky pools along the Baltic coasts of Sweden and Finland (Brinck, 1943; Lindberg, 1930), with some Norwegian records extending through the Skagerrak to the southern Atlantic coast (Nilsson, Holmen, 1995). Chromosomal confirmation of these western records would be useful. More problematic is the status of *S. griseostriatus strandi* Brinck 1943, from coastal areas in the extreme north of Norway and adjacent regions of Russia. The aedeagus, elongate and slightly truncate apically, and the more elongate parameres, suggest that it belongs to *S. griseostriatus* rather than *S. multilineatus*, but the beetle is larger than either of these species, and its morphology is also rather different (Brinck, 1943). It seems very possible that chromosome studies might show this to be a separate species.

Lindberg (1930) regarded material from Siberia as *S. multilineatus*, while he recorded both *S. multilineatus* and *S. griseostriatus strandi* (as v. *maritimus*) from Kamchatka. He recorded material from the Pyrenees, Corfu and Syria as a form of *S. griseostriatus*. The number of species now found in central Europe alone is sufficient to indicate that these broader conclusions cannot be sustained. There does, however, remain the problem of various named forms from different parts of the range of the complex. Ribera (2003), on the basis of mitochondrial DNA studies, showed that most North American material was very distinct from European, and in the light of these findings it seems likely that the various names applied to Nearctic material are not applicable to the species discussed here. More problematic are three names used for material from Europe and the Middle East. Georgiev (1959) described *S. macedonicus* from the Šar Planina mountains of Macedonia, near the Albanian border. This species was noted for its small size (3.8 mm), and was not listed as Bulgarian by Georgiev (1987). *S. riberae* is clearly a larger beetle, and, in view of the abrupt geographical changes between the ranges of various species in the Alps, some specimens of which may be as small as *S. macedonicus,* it is likely that *S. macedonicus* is either another species whose karyotype is as yet unknown, or one of the Alpine species at the extreme east of its range. There are two *S. griseostriatus*-group names referring to Middle Eastern material. *S. piochardi* Regimbart, 1877, from Djebel es Sheikh (Mt Hermon) is certainly a member of this group (R.B.A. has seen a syntype), and *S. palaestinus* Baudi, 1894 is very probably from the same area – the only mountains high enough in the area! It is just possible that these names could apply to *S. creticus*. At present this species has a very small isolated distribution on the Omalos plateau in western Crete. However, there is one Cretan Dytiscid, *Nebrioporus stearinus* Kolenati 1845, whose distribution includes both Crete and the Transcaucasus (Zaitzev, 1953). The Transcaucasian fauna is related to that of the Mt Hermon range. It would certainly interesting to get living material from Mt Hermon.

One thing which should be borne in mind is that chromosomal studies such as this cannot give information about the closeness of relationship between different species – they reveal karyotype differences which may indicate that populations belong to different species. There is some information on the phylogeny of some of the species, stemming from the ongoing research on mitochondrial DNA by Ignacio Ribera. The separation of American and European populations (Ribera, 2003) has already been mentioned. However, he has informed us (in litt. 17.VII.2006) that within the European material the deepest split is between a Bulgarian-Turkish clade and the rest of the material, from western and central Europe, and North Africa. At present the differences between various western European populations are not apparent. The chromosomal data reported here may be useful in focusing some of the future DNA work.

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