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The karyotypes of *Berosus* (s.str.) *affinis* BRULLE and *B.* (s.str.) *hispanicus* KÜSTER, with notes on the egg cocoons (Coleoptera: Hydrophilidae)

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Abstract

The karyotypes of *Berosus* (s.str.) *affinis* BRULLE and *B.* (s.str.) *hispanicus* KÜSTER are illustrated and compared. Altough very similar, with 8 pairs of autosomes and sex chromosomes which are XYp (male), XX (female), the two species differ in the smaller autosomes 7 and 8, and X-chromosome, of *B. hispanicus*. The interpretation of the karyotype of *B. affinis* given by SHAARAWI & ANGUS (1991) is corrected, and no difference can now be found between the karyotypes of *B. affinis* and *B.* (s.str.) *luridus* (L.). B-chromosomes were found in one population of *B. affinis*. No difference has been found between the chromosomes of "normal" *B. affinis* and specimens referred to *B. affinis* var. *algericus* KUWERT. A discussion is given of problems resulting from uneven condensation of the chromosomes in some preparations.

The egg cocoon of *B. hispanicus* is shown to be slightly different from those of *B. affinis* and *B. luridus*, and the cocoons of these three species are completely different from those of *B.* (s.str.) *signaticollis* CHARPENTIER, *B.* (*Enoplurus*) *fulvus* KUWERT and *B.* (*Enoplurus*) *bispina* REICHE & SAULCY.

Key words: Hydrophilidae, *Berosus*, chromosomes, egg cocoons

Introduction

The publication by AOUAD & AGUESSE (1991) on the Moroccan representatives of the *Berosus* (s.str.) *affinis* BRULLE group suggested that three species were involved: *B. affinis* itself, mistakenly referred to as *B. suturalis* KÜSTER as a result of mistaken interpretation of a museum label; *B. hispanicus* KÜSTER, mistakenly referred to as *B. affinis* as a result of the same misinterpretation; and *B. algericus* KUWERT. The distinction between *B. affinis* and *B. hispanicus* is abundantly clear, but, notwithstanding the apparent aedeagal differences figured by AOUAD & AGUESSE (1991), the senior author was unable to find any consistent difference between *B. affinis* and *B. algericus*. The pronotal distinguishing character, though clear, is inconsistent, while the aedeagal character (published by AOUAD & AGUESSE 1991) could not be detected, even with the scanning electron microscope. This result is consistent with the findings of SCHÖDL (1993), who placed *B. algericus* in synonymy with *B. affinis*, and also corrected the nomenclature.

However, chromosomal investigations by Angus and Aouad in July 1991 at first appeared to support the idea that *B. affinis* and *B. algericus* were separate species: all karyotypes of *B. algericus* showed the X-chromosome to be as small as the smallest autosomes (pairs 7 and 8), quite unlike the arrangement figured by SHAARAWI & ANGUS (1991: Figs. 1, 2) for *B. affinis* (from Greece and England). This work soon revealed a further problem: karyotypes obtained from Moroccan specimes of "typical" *B. affinis* also showed the small X-chromosome, and resembled those obtained for *B. algericus* rather than the published *B. affinis*. It therefore became necessary to reinvestigate the karyotype of *B. affinis*.

Material and Methods

The sources of the material studied, and the tissues used, are given below.

SPECIES	LOCALITY OF ORIGIN	TISSUES USED
B. affinis	England: Somerset, Steart	Embryo, mid-gut, testis
	England: Sussex, Cuckmere Haven	Mid-gut, testis
	Greece: Corfu	Embryo
	Morocco: Fes	Mid-gut, testis
	Morocco: Rabat	Embryo, mid-gut, testis
B. affinis var. algericus	Morocco: Fes	Mid-gut, testis
	Morocco: Rabat	Embryo, mid-gut, testis
B. hispanicus	Morocco: Fes	Mid-gut, testis
	Morocco: Rabat	Embryo, mid-gut, testis

The methods of preparation are given by SHAARAWI & ANGUS (1991). In addition, the Relative Chromosome Lengths of the various chromosomes (the length of each chromosome as a percentage of the total haploid autosome length) are given in Table 2, and the Centromere Indices of the chromosomes (the length of the shorter arm of each chromosome as a percentage of the total length of the chromosome) are given in Table 3. Both these parameters are subject to experimental error because of irregularities in chromosome condensation, and *Berosus*, with its relatively long chromosomes, appears particularly prone to this problem. Where the uneven condensation affects distinctive chromosomes (e.g. autosomes 1-3), the use of average values with relevant statistics deals with the problem. However, where different chromosome pairs are of similar size and shape, correct attribution of individual chromosomes is unlikely to be possible in all cases. Here, because the sizes of the chromosomes are so similar, the values obtained for Relative Chromosome Length will not be altered perceptibly by the errors. However, where only a few preparations are available, mistaken interpretations may occur (see later). With Centromere Index a further complication arises: the Index is calculated from measurements of the short arm and the whole chromosome, but if the arms are almost the same length, then irregular condensation will mean that, in a given chromosome pair, it is not always the same arm which appears shorter. Unless there is some feature of the arm, apart from its size, by which it can be recognized, the problem is insoluble and will result in the centromere index of metacentric chromosomes appearing too low. As an indication of this problem, Table 3 gives not only the mean values and 95 % confidence limits, but also the observed range of values and the number of cases where the measurement was 50. Where a substantial proportion of the observed values are at 50, then it must be assumed that some should have been greater than 50, and the Centromere Index should be closer to 50 than is given.

Berosus affinis (excluding var. *algericus*)

Karyotypes are shown in Figs. 1 - 5. The X-chromosome is approximately the same length as autosome pairs 7 and 8, though apparently a little larger than these, but always clearly shorter than pairs 4, 5 and 6. It may not always be possible to distinguish individual replicates of chromosomes 7, 8 and X. Figs. 3 and 4 are of preparations from embryos of Corfu material, with Fig. 3 being the preparation misinterpreted by SHAARAWI & ANGUS (1991). Fig. 4 clearly represents the same arrangement as English (Figs. 1 and 3) and Moroccan (Fig. 5) material. Fig. 3 suffers from a mismatch in pair 8, where the longer replicate can be seen to be less condensed than the shorter one. SHAARAWI & ANGUS (1991) used a lighter print of this preparation, to enhance traces of apparent G-banding, and this emphasised the mismatches, and led to the

misinterpretation. It should be noted that the mismatch in chromosome 8, which would make the present interpretation unsafe if based only on this preparation, is no worse than the mismatch in chromosome 3, where the chromosome is so distinctive that the mismatch is obvious.

The Moroccan material (Fig. 5) is similar to that from other localities. One English population, from Steart (Somerset) included a specimen with one or two B-chromosomes (Fig. 2). Although no C-banding was attempted, the B-chromosomes always appeared more condensed than the others, and are probably heterochromatic.

Chromosome	*B. affinis*	*B. hispanicus*
1	24.73 (N = 19) 24.04 - 25.42	25.15 (N = 8) 24.28 - 26.02
2	14.73 (N = 20) 14.37 - 15.09	17.46 (N = 8) 16.93 - 17.99
3	13.59 (N = 20) 13.23 - 13.94	14.16 (N = 8) 13.55 - 14.77
4	11.27 (N = 20) 10.96 - 11.58	11.28 (N = 8) 10.88 - 11.67
5	10.37 (N = 20) 10.13 - 10.61	10.11 (N = 8) 9.58 - 10.64
6	9.78 (N = 20) 9.51 - 10.05	9.16 (N = 8) 8.85 - 9.48
7	7.94 (N = 20) 7.74 - 8.13	6.49 (N = 8) 6.08 - 6.89
8	7.76 (N = 20) 7.75 - 8.00	6.21 (N = 8) 5.69 - 6.74
X	8.00 (N = 10) 7.67 - 8.33	6.32 (N = 5) 5.67 - 6.97

Table 2: Relative Chromosome Length. Mean, number of replicates and 95 % Confidence Limits.

Berosus affinis var. *algericus*

This is taken as Moroccan specimens in which the dark mark on the pronotal disc shows no diminution in the middle, and there is no reduction in dense puncturation in the mid line. A very regularly condensed preparation from testis is shown in Fig. 6. The apparent widening of the centromeric constriction in one replicate of chromosome 7 is an artifact resulting from a thin strand of non-staining material lying over this part of the chromosome. This karyotype shows no difference from those obtained from "normal" *B. affinis*, and supports the conclusion that *B. algericus* is simply a variety of *B. affinis*.

Comparison of the karyotypes of *B. affinis* and *B. luridus* (L.): The present reassessment of the karyotype of *B. affinis*, and study of additional material of *B. luridus* (from Thompson Common, Norfolk), shows that the slight differences between the karyotypes suggested by SHAARAWI &

ANGUS (1991) are not valid, but merely reflect individual variation due to irregular condensation of the chromosomes in both species. However, the morphological and distributional differences between these two species preclude any suggestion that they are conspecific.

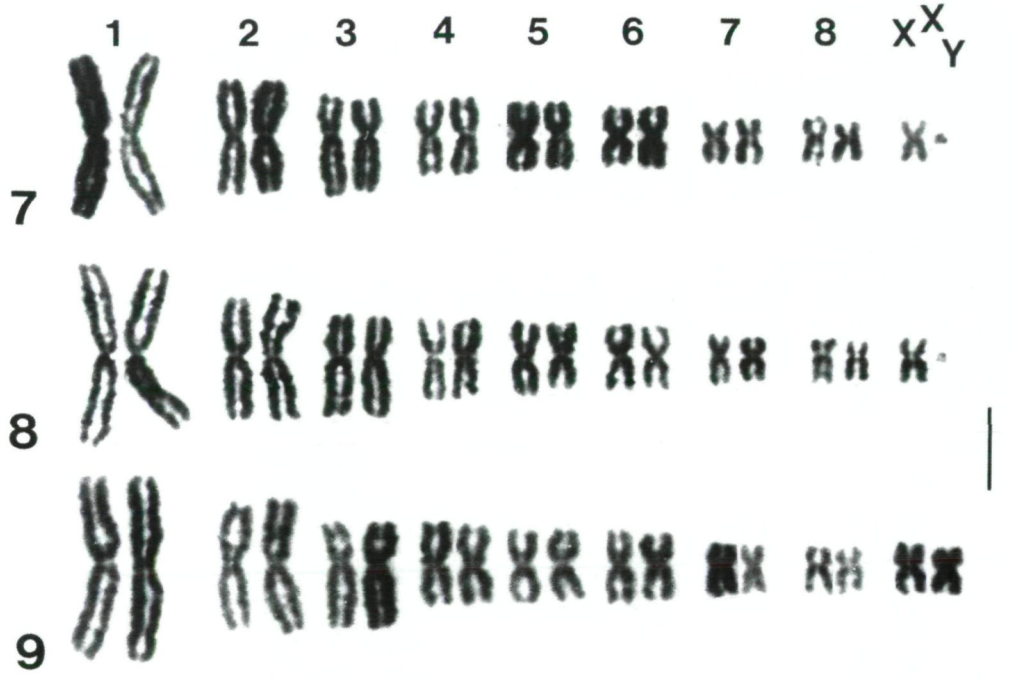
Berosus hispanicus

Karyotypes of Moroccan *B. hispanicus* are shown in Figs. 7 - 9. Although similar to those of *B. affinis*, they are clearly distinct because of the smaller X-chromosome and autosome pairs 7 and 8. This is immediately apparent from examination of the figures, and is supported by the Relative Chromosome Lengths given in Table 2. This chromosomal difference is particularly striking in view of the similarity of the karyotypes of *B. affinis* and *B. luridus*.

Chromosome	*B. affinis*	*B. hispanicus*
1	49.41 (N = 19) 48.76 - 50.06 48.3. - 50 (14)	48.71 (N = 8) 47.34 - 50.08 45.70 - 50 (4)
2	48.67 (N = 20) 48.07 - 49.25 47.4 - 50 (7)	47.81 (N = 8) 46.80 - 48.83 45.50 - 50 (1)
3	38.52 (N = 20) 37.70 - 39.33 36.40 - 41.70	38.13 (N = 8) 36.80 - 39.44 36.80 - 39.50
4	46.27 (N = 19) 45.08 - 47.46 44.10 - 50 (3)	48.30 (N = 8) 46.77 - 49.83 46.40 - 50 (4)
5	46.78 (N = 20) 45.73 - 47.82 42.90 - 50 (4)	48.20 (N = 8) 45.92 - 50.48 42.80 - 50 (5)
6	46.25 (N = 20) 45.18 - 47.31 40.00 - 50 (3)	47.91 (N = 8) 46.04 - 49.79 45.50 - 50 (4)
7	45.16 (N = 20) 44.25 - 46.05 39.3 - 46.70	42.65 (N = 8) 40.52 - 44.78 37.50 - 44.40
8	47.37 (N = 20) 46.27 - 48.46 42.90 - 50 (7)	36.83 (N = 8) 31.44 - 42.21 31.30 - 44.40
X	45.60 (N = 10) 44.01 - 47.19 41.70 - 50 (1)	43.38 (N = 5) 38.89 - 47.87 37.50 - 47.10

Table 3: Centromere Index. Mean, number of replicates, 95 % Confidence Limits and Observed Range, with number of replicates giving a C.I. of 50.

Figs. 1 - 6: *Berosus affinis*, mitotic chromosomes of males. 1) embryo, Cuckmere Haven, Sussex; 2) testis with 2 B-chromosomes, Steart, Somerset; 3, 4) embryos, Corfu, Greece; 5) mid gut with the Y-chromosome lost, Fes, Morocco; 6) *B. affinis* var. *algericus*, testis, Fes, Morocco. The scale line represents 5 μm.



Figs. 7 - 9: *Berosus hispanicus*, mitotic chromosomes from embryos, Rabat, Morocco. 7, 8) males; 9) female. The scale line represents 5 μm.

Egg cocoons

In the course of this work living *Berosus* were routinely kept in aquaria, and at various times all the species laid eggs in their characteristic silk cocoons. *B. affinis*, *B. luridus* and *B. hispanicus* lay their eggs singly, in cocoons of spun silk attached to underwater vegetation. The eggs are elongate, 0.8 - 1.0 mm long and about 0.3 mm wide. Beneath the egg is a silk ribbon, as wide as the egg and extending 1 - 1.5 mm beyond one end of the egg. On each side of the egg is a sheet of silk, flat ventrally (where it is continuous with the basal ribbon), and curved above, so that it resembles a slice from the top of a large circle, with its depth approximately half the redius of the circle. The top of the cocoon is formed from a second narrow ribbon of silk, extending from the back of the basal ribbon, over the curved tops of the side pieces, and then fused with the projecting part of the basal ribbon. In *B. affinis* (including var. *algericus*) and *B. luridus*, the sides of the cocoon are vertical and parallel, but in *B. hispanicus* they are curved outwards medially and also diverge towards the top, giving a somewhat boat-like shape. The top ribbon of the *B. hispanicus* cocoon is thus wider than the bottom ribbon over the middle part of the egg.

It may be noted that these egg cocoons differ from those of *B. signaticollis* CHARPENTIER, *B. fulvus* KUWERT and *B. bispina* REICHE & SAULCY, where two or three eggs are enclosed between oval discs of silk, which frequently project as ribbons at one end of the egg bag.

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chromosomes for her Ph.D. degree at Royal Holloway, and we thank Royal Holloway for providing the facilities to carry out this research in the Department of Biology, including the visit by Dr. Aouad in 1991.

Zusammenfassung

Die Chromosomen von *Berosus* (s.str.) *affinis* BRULLE und *B.* (s.str.) *hispanicus* KÜSTER wurden untersucht und miteinander verglichen. Die Chromosomen der beiden Arten sind einander sehr ähnlich, mit 8 Paaren von Autosomen und XYp (♂) bzw. XX (♀) Geschlechtschromosomen. Sie unterscheiden sich nur durch die Größe der Autosomen 7 und 8 und das X-Chromosom. Die Interpretation der Chromosomen von *B. affinis* durch SHAARAWI & ANGUS (1991) wird berichtigt. Es konnte kein Unterschied zwischen den Chromosomen von *B. affinis* and *B.* (s.str.) *luridus* (L.) gefunden werden. B-Chromosomen konnten in einer Population von *B. affinis* nachgewiesen werden. Keinerlei Unterschiede konnten zwischen den Chromosomen von "normalen" *B. affinis* und *B. affinis* var. *algericus* KUWERT festgestellt werden. Das Problem ungleichmäßiger Kondensierungen in Chromosomen-Präparaten wird diskutiert.

Die Eikokons von *B. hispanicus* unterscheiden sich geringfügig von den Eikokons von *B. affinis* und *B. luridus*, während sich jene von *B.* (s.str.) *signaticollis* CHARPENTIER, *B.* (*Enoplurus*) *fulvus* KUWERT und *B.* (*Enoplurus*) *bispina* REICHE & SAULCY deutlich von den 3 genannten Arten unterscheiden.

References

AOUAD, N. & P. AGUESSE, 1991: Notes sur les *Berosus* du groupe *affinis* BRULLE, 1835, du Maroc (Coleoptera, Hydrophilidae). - Nouv. Revue Ent. (N.S.) 8 (1): 77 - 84.

SCHÖDL, S. 1993: Revision der Gattung *Berosus* LEACH. 3. Teil: Die paläarktischen und orientalischen Arten der Untergattung *Berosus* s.str. - Koleopt. Rdsch. 63: 189 - 233.

SHAARAWI, F.A.I. & R.B. ANGUS 1991: Chromosomal analysis of some European species of the genus *Berosus* LEACH (Coleoptera: Hydrophilidae). Koleopt. Rdsch. 61: 105 - 110.

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