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Chromosomal confirmation of the species rank of *Notonecta meridionalis* Poisson, 1926 (Heteroptera: Notonectidae)

Хромосомные признаки, подтверждающие статус *Notonecta meridionalis* Poisson, 1926 (Heteroptera: Notonectidae)

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ABSTRACT. C-banded karyotypes obtained from Spanish material show that *Notonecta meridionalis* Poisson is a species distinct from both *N. glauca* Linnaeus and *N. obliqua* Thunberg. The Relative Chromosome Lengths and C-banding patterns of the X chromosomes of the three species are all distinct from one another, and there are also clear differences in some of the autosomes.

РЕЗЮМЕ. Кариологический анализ, проведённый методом С-бэндинга на материале из Испании, показал, что *Notonecta meridionalis* Poisson отличается от *N. glauca* Linnaeus и *N. obliqua* Thunberg. Эти три вида различаются по относительной длине хромосом, рисунку С-бэндинга Х-хромосом, а также имеют чёткие различия в некоторых аутосомах.

Introduction

The clear interspecific differences shown by the C-banded karyotypes of the four British species of *Notonecta* [Angus et al., 2004] provided a graphic illustration of the use of C-banding in identification of individual holocentric chromosomes. On receipt of a reprint of this paper, I. M. Kerzhner [*in litt.*, 29 & 30.iii.2004] wrote "There are several interesting problems in the taxonomy of European *Notonecta*, especially in the group *N. glauca – N. meridionalis – N. obliqua*." and "Poisson speculated that *N. glauca* and *N. obliqua* are good species in northern France, but successfully hybridise in southern France." He then went on to draw my attention to Aukema & Rieger's Catalogue of Palaearctic Heteroptera [Aukema & Rieger, 1995] as well as the seminal papers by Kanyukova [1973], where *N. meridionalis* is shown to be a clearly recognisable taxon which she placed as a subspecies of *N. glauca*, and Zimmermann [1982], who regarded *N. meridionalis* as a valid species. Aukema & Rieger noted the disagreement among various authors as to the status of *N. meridionalis*, and it was with this in mind that Izya Kerzhner encouraged me to investigate the chromosomes of this species if I got the chance to do so. A collecting trip to Spain in February 2004 provided this chance, and it is a great pleasure to be able to report the results in a volume published in honour of Izyaslav Moiseevich Kerzhner as well as of Aleksandr Fedorovich Emelyanov, whom I also met when I worked in the Zoological Institute, Leningrad, in 1969–1970.

Material and Methods

The *N. meridionalis* were collected in a pond for watering livestock, about 22 km SW of Almonte in the Provincia de Huelva, Spain, on February 15th, 2004. Chromosome preparations were obtained from one male and five females. The colouration of the specimens ranged from as dark as *N. obliqua* to as light as more mottled specimens of British *N. glauca*. The specimens were brought home alive and placed in aquaria with living newts (*Triturus helveticus*) (Amphibia) as food, to stimulate cell division in the mid-gut epithelium. However, they did not feed so were processed anyway. Chromosome preparations were obtained from mid-gut and ovary, as described by Angus et al. [2004]. C-banding was attempted on 2-day old slides, and although success was sporadic, the banding patterns, when obtained, were consistent. Relative Chromosome Lengths (RCL) values were calculated and are shown in Table 1. RCL is the length of each chromosome expressed as a percentage of the total haploid autosome length (i.e. excluding the sex chromosomes) in the nucleus. Its use compensates for variation in length due to different degrees of chromosome condensation in different preparations. Because the smaller chromosomes are virtually impossible to distinguish from one another, they are grouped in batches, as done by Angus et al. [2004].

Table 1. Relative Chromosome Lengths (RCL) of *Notonecta* chromosomes: means, 95% confidence limits, number of observations.

Таблица 1. Относительная длина хромосом (RCL) клопов рода *Notonecta*: среднее, 95% доверительный интервал, число наблюдений.

Chromosome	*N. meridionalis*	*N. glauca*	*N. obliqua*
1	34.1 (31 – 37.3) N = 10	26.7 (24.7 – 28.8) N = 12	23.8 (22.6 – 24.9) N = 12
2	18.9 (18.6 – 19.6) N = 10	15.9 (14.8 – 17.) N = 12	19.9 (19.0 – 20.7) N = 12
3	11.1 (10.6 – 11.6) N = 10	12.3 (11.3 – 13.2) N = 12	11.4 (10.9 – 11.9) N = 12
4 – 6	4.6 (4.2 – 4.9) N = 30	6 9 (6.6 – 7.1) N = 36	7.5 (7.1 – 7.8) N = 36
7 – 9	3.1 (2.2 – 3.8) N = 27	5 7 (5.5 – 5.9) N = 36	5.4 (5.1 – 5.7) N = 35
10	3.1 (2.4 – 3.8) N = 10	3 8 (3.5 – 4.0) N = 12	3.2 (2.6 – 3.8) N = 12
11	2.5 (1.7 – 3.2) N = 8	3 2 (2.7 – 3.7) N = 12	2.6 (2.2 – 2.9) N = 12
12		2.2 (1.5 – 2.9) N = 6	
X	22.6 (20.0 – 25.2) N = 8	21.3 (19.1 – 23.6) N = 8	16.7 (15.0 – 18.3) N = 9
Y	11.4 (unavailable) N = 2	10.5 (9.3 – 11.7) N = 4	8.5 (6.0 – 10.5) N = 3

Results

Representative karyotypes are shown in Fig. 1, a–d and selected karyotypes of *N. glauca* and *N. obliqua* (originally figured by Angus et al. [2004]) are shown in Fig. 1, e – h, for comparison. In some cases one or two small autosomes are missing from the karyotypes. This results from damage during the preparation and does not represent natural variation, but the preparations are figured as they give the clearest illustrations of the main chromosomal features. Relative Chromosome Length (RCL) data are given in Table 1, with the data for *N. glauca* and *N. obliqua* taken from Angus et al. [2004].

In *N. meridionalis* autosome 1 is characterised by concentrations of heterochromatin towards the ends of the chromosome, these appearing as multiple bands in less condensed chromosomes (Fig. 1, b – d), but as single blocks in more condensed chromosomes (Fig. 1 a). The heterochromatic block in the lower portion of the chromosome (using the orientation shown in Fig. 1) is larger than that in the upper portion. The general appearance of this chromosome is very similar to autosome 1 of *N. glauca* (Fig. 1, e, f), though the RCL of *N. meridionalis* autosome 1 is significantly greater than that of *N. glauca* (Table 1). The C-banding arrangement of autosome 1 of *N. obliqua* (Fig. 1, g, h) is in general similar, but appears less prominent in the preparations obtained. The RCL of this chromosome is also smaller (Table 1).

Autosome 2 of *N. meridionalis* is little more than half the length of autosome 1 (Table 1), and distinctly smaller than the X chromosome. There is a fairly distinct subterminal C-band in the upper part of the chromosome, while the lower end is somewhat darker in some preparations. The size ratios of autosomes 1 and 2 in *N. glauca* are similar to those of *N. meridionalis*, (Table 1) but in *N. glauca* there are two very distinct C-bands, one at each end of the middle third of the chromosome (Fig. 1, e, f). Autosome 2 of *N. obliqua* is more similar in length to autosome 1 (Table 1), and has weak terminal C-bands (Fig. 1, g, h).

Autosome 3 of *N. meridionalis* has a distinct submedian C-band (Fig. 1, a – d), a feature shared with autosome 3 of *N. glauca* (Fig. 1, e, f), but not with *N. obliqua*, where autosomes 3 and 4 have distinct terminal or subterminal C-bands (Fig. 1, g, h).

The remaining autosomes do not show any sufficiently distinctive features to allow them to be either identified individually or to be compared with those of the other species. In some specimens of *N. glauca* there is an additional pair of small autosomes (Fig. 1, f).

The X chromosome of *N. meridionalis* is the second longest in the nucleus (Table 1), and has a strong heterochromatic block at one end (the lower end in the orientation used here) (Fig. 1, a – d), while the upper end may also be somewhat darkened and in some preparations (Fig. 1, b) there is a suggestion of a weak median C-band. In less condensed chromosomes the lower heterochromatic block may appear divided (Fig. 1, d). The X chromosome of *N. glauca* (Fig. 1, e, f) has an extensive C-banding pattern, quite unlike that of *N. meridionalis*. The X chromosome of *N. obliqua* is smaller than autosome 2 (Table 1), with a distinct C-band at each end and a smaller band in the middle (Fig. 1, g, h).

The Y chromosomes of the three species appear very similar in both size and in being largely heterochromatic. Only two observations are available for *N. meridionalis* and these do not allow calculation of the 95% confidence limits for its RCL.

Discussion

The C-banded chromosomes of the three species considered here provide a number of very clear features enabling their karyotypes to be distinguished from one another. The heavily C-banded X chromosome and the pair of submedian C-bands on autosome 2 of *N. glauca* are features not shown by either of the other two species. The rather similar lengths of autosomes 1 and 2 and the X chromosome of *obliqua* are very distinctive, as are the terminal C-bands on autosomes 3 and 4. There is thus no way the karyotype of *N. meridionalis* could be confused with those of the other two species. The differences in

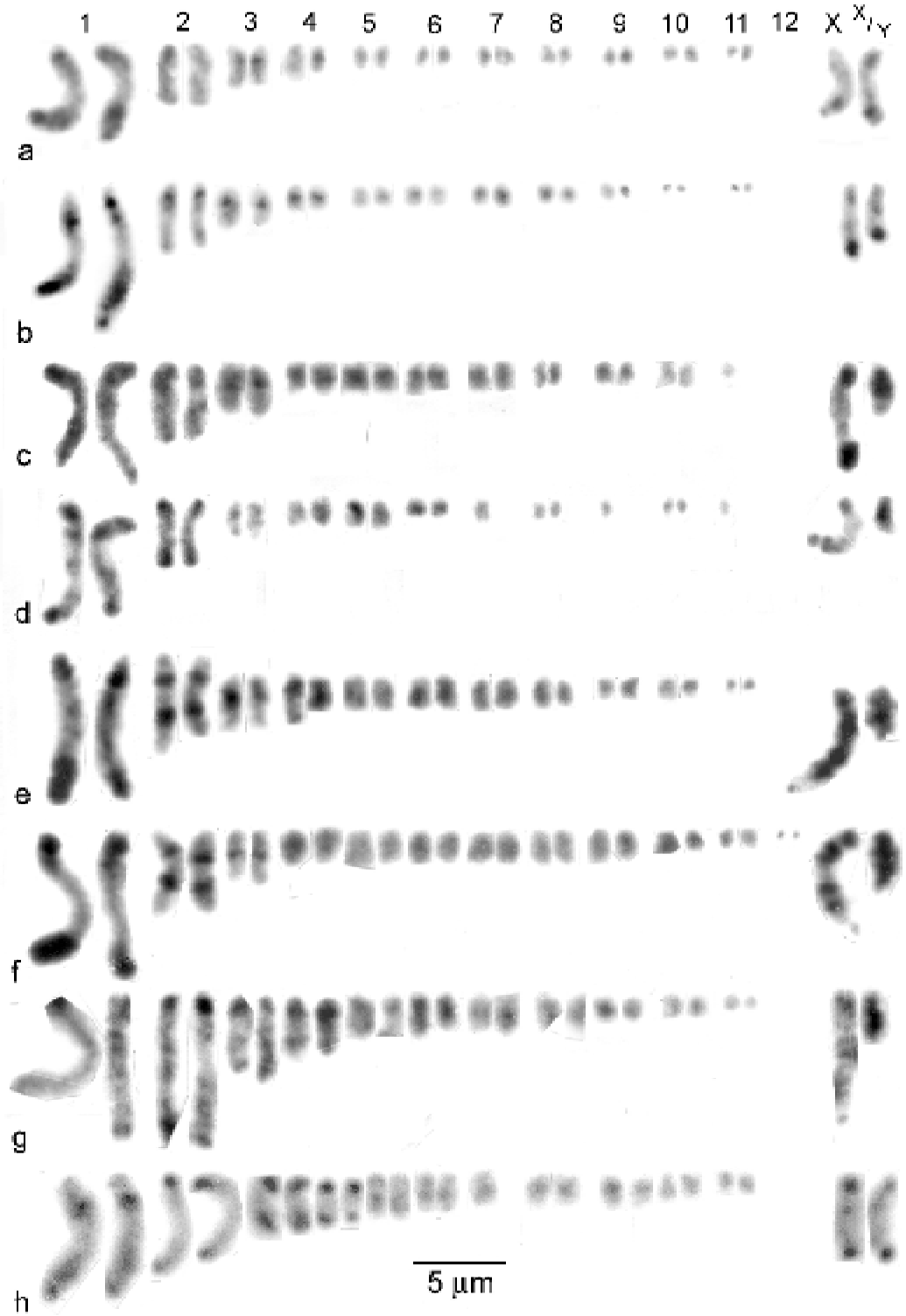

Fig. 1. C-banded karyotypes of *Notonecta* spp.: a, b — *N. meridionalis*, ♀, ovary; c, d — *N. meridionalis*, ♂, mid-gut (some small chromosomes are missing from these preparations); e, f — *N. glauca*, ♂, mid-gut, from Surrey, England (f has one extra pair of small autosomes); g — *N. obliqua*, ♂, mid-gut, from Surrey, England; h — *N. obliqua*, ♀, ovary, from Surrey, England (one autosome is missing from this preparation).

Рис. 1. С-бэндинг в кариотипе *Notonecta* spp.: a, b — *N. meridionalis*, ♀, яичник; c, d — *N. meridionalis*, ♂, средняя кишка (на препарате отсутствуют некоторые мелкие хромосомы); e, f — *N. glauca*, ♂, средняя кишка, из Суррея, Англия (f имеет одну дополнительную пару небольших аутосом); g — *N. obliqua*, ♂, средняя кишка, из Суррея, Англия; h — *N. obliqua*, ♀, яичник, из Суррея, Англия (на препарате отсутствует одна аутосома).

the sequence of RCL values obtained for the larger chromosomes of all three species means that there must be translocational differences between them. Thus the individual autosome pairs are not entirely homologous in the three species, because sections of chromosome have been transferred between different autosomes. This would make the chromosomes of any hybrids unable to pair up at meiosis, and hence result in hybrid sterility.

The conclusion has to be that the extent and nature of the differences between the karyotypes of these three species leaves no doubt of their status as distinct species.

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