

## Achiasmatic male meiosis in three *Micronecta* species (Heteroptera: Nepomorpha: Micronectidae)

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**Abstract.** Male meiosis of three water boatmen species *Micronecta* (*Dichaetonecta*) *scholtzi* (Fieber, 1860), *M. (Micronecta) poweri* (Douglas et Scott, 1869) and *M. (Micronecta) griseola* Horvath, 1899 (Corixoidea: Micronectidae) was studied paying special attention to the presence or absence of chiasmata. Condensation stage revealed 11 autosomal bivalents consisting of side by side associated homologous chromosomes. At this stage positively heterochromatic X and Y chromosomes were attached to the nucleolus. There were no m-chromosomes. The chromosome number of all species is  $2n=22+XY$ . Side by side association of homologous chromosomes was still seen at late condensation stage. At prometaphase and metaphase I, the bivalents oriented parallel to the equatorial plane and homologous chromosomes are facing opposite poles. Hence, male meiosis in these species is achiasmatic of alignment type. At metaphase II chromosomes showed variable positions in the plate. In *M. scholtzi*, the Y chromosome is smaller than the X chromosome, while in *M. poweri* and *M. griseola* X and Y chromosomes were similar in size. It is suggested that achiasmatic male meiosis and lack of m-chromosomes are cytogenetic features separating the family Micronectidae from the family Corixidae among the superfamily Corixoidea within the infraorder Nepomorpha. It seems plausible that achiasmatic male meiosis has emerged in Heteroptera more than once during their evolution.

**Key words:** Heteroptera, Micronectidae, achiasmatic male meiosis.

### INTRODUCTION

Most often during meiosis, chiasma formation ties homologous chromosomes together until their separation in the reductional division. However, in some groups of animals chiasma formation during meiosis is replaced by other, achiasmatic means. Achiasmatic meiosis, as a rule, is restricted to the heterogametic sex only, and has been described in different groups of animals (White, 1973).

In Hemiptera, achiasmatic meiosis has been found both in Homoptera (Schrader, 1931; Nur, 1965; Blackman, 1976; Hales, 1989) and in six families of Heteroptera, belonging to two infraorders: Leptopodomorpha and Cimicomorpha (Nokkala, Nokkala, 1983; Nokkala, Nokkala, 1984; Nokkala, Nokkala, 1986a, b; Nokkala, Grozeva, 2000; Grozeva, Nokkala, 2002). Recently, achiasmatic meiosis had been reported for the first time in the infraorder Nepomorpha (Ituarte, Papeschi, 2004). Ac-

ording to previous cytogenetic reports on 28 species of family Corixidae (Corixoidea) 27 species display pre-reductional chiasmatic meiosis and a stable chromosome formula  $2n=24(20+2m+XY)$  (except in *Cymatia bondorffi* (Sahlberg, 1819),  $2n=26$ ) (Ituarte, Papeschi, 2004; Bressa, Papeschi, 2007).

Fieber (1851) was the first who considered the Micronectidae as a separate taxon Sigarae. Subsequent authors did not recognize the family or subfamily status of Micronectidae, e.g., Puton (1880), Oshanin (1912). Jaczewski (1924) introduced the modern name Micronectinae as a subfamily of Corixidae, and this status was used by nearly every author. Recently, Nieser (2002) raised the taxon to family status: Micronectidae. Over 150 species are described in the family worldwide; but over 100 of them belong to the genus *Micronecta*.

According to Nieser, Chen (2006) the Micronectidae consists of two subfamilies: the monotypic Synaptogobiinae (described from Brazil) and Micronectinae with four genera (*Micronecta* Kirkaldy, 1897 from the Old World, *Synptonecta* Lundblad, 1933 from SE Asia and Florida, *Monogobia* Nieser et Chen, 2006, and *Tenagobia* Bergroth, 1899 from Tropical and Neotropical America). Except for two species, there are no cytogenetic data on the Micronectidae. The chromosome formula of *Micronecta poweri* is reported by Southwood, Leston (1959) as  $2n=24(22+XY)$ ; *Tenagobia (Fuscagobia) fuscata* (Corixoidea, Micronectidae) has  $2n=30(28+XY)$  and the male meiosis is achiasmatic (Ituarte, Papeschi, 2004). The information available on cytotaxonomy of the family Micronectidae are limited to these data. The spermatogenesis of the *Micronecta* species has not been studied earlier.

In the present paper, the spermatogenesis of *Micronecta (Dichaetonecta) scholtzi* (Fieber, 1860), *Micronecta (Micronecta) poweri* (Douglas, Scott, 1869) and *M. (Micronec-*

*ta) griseola* Horvath, 1899 was studied paying special attention to the presence or absence of chiasmata.

## MATERIAL AND METHODS

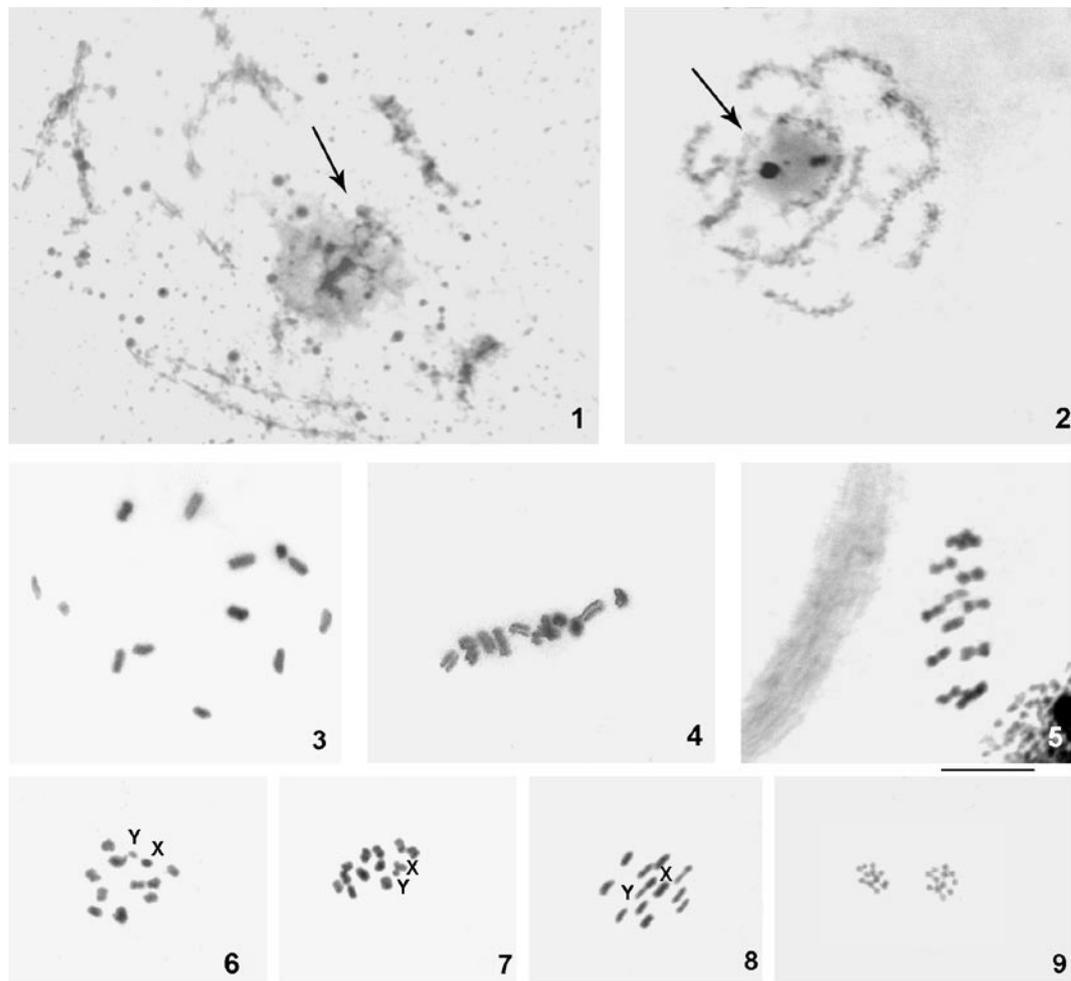
A number of males of three *Micronecta* species – 49 males of *Micronecta (Dichaetonecta) scholtzi*, 6 males of *M. (Micronecta) poweri* and 8 males of *M. (Micronecta) griseola* – were collected in different water pools and rivers in Bulgaria and fixed in 3:1 fixative (96% ethanol - glacial acetic acid mixture). The abdomens were dissected in 45% acetic acid and the reproductive system structure was analysed under stereoscope. Then the gonads were squashed in a small drop of 45% acetic acid. The cover slips were removed by dry ice technique. Slides were dehydrated in fresh fixative (3:1) and air dried. To study the number and the behaviour of the chromosomes the preparations were stained by Schiff-Giemsa method after Grozeva, Nokkala (1996).

Chromosome spreads were analysed using a Laborlux 12 (Leitz, Wetzlar, Germany) microscope with Olympus C 5060 digital camera.

## RESULTS

The male reproductive system of the three species was found to coincide with that described and drawn for *Nerthra terrestris* (Kevan, 1948) (Nepomorpha: Gelastocoridae) by Pendergrast (1957) as *Mononyx*: each testis comprises two lobes, their apical parts are snail-coiled and the long thick uncoiled strap-like part crosses the abdomen to give rise to the vas deferens. It is an irregularly-coiled duct. Ducts from a pair of accessory glands enter the apex of the long ductus ejaculatorius.

The number of chromosomes and their behaviour of all species are quite identical and will be presented together. The adult males



**Figs 1-9.** Male meiosis of *Micronecta* species. **1, 2** - early condensation stages of *M. scholtzi*. **3** - late condensation stage of *M. scholtzi*, showing 13 chromosome elements (11+XY). **4** - prometaphase I of *M. scholtzi* in side view. The bivalents consist of two side by side aligned chromosomes facing the opposite poles. **5** - metaphase I of *M. griseola* in side view. **6, 7** - second prometaphase (PMII) of *M. scholtzi*. The Y chromosome is clearly smaller than the X chromosome, and the sex chromosomes can be seen separately (**6**) or together (**7**). **8** - second metaphase (MII) of *M. griseola*, the sex chromosomes are quite similar in size, forming pseudobivalent in the middle of the plate. **9** - second anaphase (AII) of *M. scholtzi*, every anaphase group contains 12 chromosomes, eleven autosomes and a sex chromosome (X/Y). Bar=10µm.

usually had the testes almost full of sperms with a small number of dividing cells per testis. No spermatogonial cells at metaphase were observed. Among the meiotic stages, post-pachytene stages were abundantly present in the testes. Nuclei at early condensation stages revealed autosomal bivalents, which

consisted of side by side aligned homologous chromosomes (Figs 1, 2), and the positively heteropycnotic X and Y sex chromosomes connected to a nucleolus (Figs 1, 2). In some cells, in addition to sex chromosomes, 11 autosomal bivalents could be traced (Fig. 2), hence haploid chromosome number in these species is

$n=11+XY$ . Similar side by side association of homologous chromosomes was still seen in bivalents at late condensation stage, revealing 13 chromosome elements ( $11+X+Y$ ) (Fig. 3) and prometaphase I (Fig. 4). In prometaphase, the bivalents lay parallel to the equatorial plane, the homologous chromosomes facing opposite poles. This orientation of bivalents was still observable in MI cells (Fig. 5). Clearly, chiasmata are absent and meiosis in males of these species is achiasmatic. At the second metaphase (MII), autosomes did not form a clear ring but showed variable position from quite even distribution in prometaphase II (Figs 6, 7) to a radial-like arrangement in MII (Fig. 8). The sex chromosomes could be seen separately (Figs 6, 7) in *M. scholtzi* or forming a pseudo-bivalent in the centre of the plate (Fig. 8) in *M. griseola*. In *M. scholtzi*, the Y chromosome is clearly smaller than the X chromosome (Figs 6, 7), while in *M. griseola* and *M. poweri* (not shown) the sex chromosomes are quite similar in size (Fig. 8). There are no m-chromosomes in the karyotype of any of these species. Consequently, in second anaphase (AII), every group contained 12 chromosomes: eleven autosomes and a sex chromosome (X/Y). Hence male meiosis is post-reductional for the sex chromosomes (Fig. 9).

## DISCUSSION

On the basis of the morphology of the internal reproductive apparatus in a number of heteropteran families, Pendergrast (1957) has identified several characters, which could provide phylogenetic indications at various taxonomic levels. He refers, in particular, to the number of seminal follicles per testis in males and the number of ovarioles per ovary in females. In many families male testes were shown to consist of 7 follicles (Pendergrast, 1957), and this number is taken as the gener-

alized or plesiomorphic condition in Heteroptera (Akingbohunge, 1983). The analysis of reproductive system of *Micronecta* males studied displayed a reduced follicle number as an apomorphy, and confirms the conclusion of Pendergrast (1957) that the reproductive organs of all aquatic bugs are of the same type.

In Heteroptera, the existence of achiasmatic meiosis has been described in seven families, belonging to three infraorders: Leptopodomorpha, in family Saldidae (Nokkala, Nokkala, 1983); two superfamilies of Cimicomorpha, Miroidea - families Miridae (Nokkala, Nokkala, 1986a) and Microphysidae (Nokkala, Grozeva, 2000); and Cimicoidea: Nabidae (Nokkala, Nokkala, 1984), Anthocoridae (Nokkala, Nokkala, 1986b), and Cimicidae (Grozeva, Nokkala, 2002); and Nepomorpha: in family Micronectidae (Ituarte, Papeschi, 2004). The achiasmatic meiosis in Heteroptera must be of very ancient origin, since some divergence has occurred in its cytological characteristics during the course of evolution (Nokkala, Grozeva, 2000). The most common type of achiasmatic meiosis is the meiosis of alignment type characterized by tight side by side alignment of homologous chromosomes throughout prophase up till MI. Meiosis of this type has been described in Saldidae (Nokkala, Nokkala, 1983), Nabidae (Nokkala, Nokkala 1984), Anthocoridae (Nokkala, Nokkala, 1986b), and Microphysidae (Nokkala, Grozeva, 2000), and has been found also in Micronectidae both in the genus *Tenagobia* (Ituarte, Papeschi, 2004) and in the genus *Micronecta* (present study). The other, collochore type of achiasmatic meiosis has been found in the families Miridae and Cimicidae (Nokkala, Nokkala, 1986a; Grozeva, Nokkala, 2002). The homologous chromosomes of these species are physically associated in one or two regions via so called collochores.

According to our observations among

the Heteroptera, if the achiasmatic meiosis is found in one species it will be found in other genera of the whole family. It seems to be a stable character on the family level. For instance, it is well proved in the Nabidae, where seven genera are examined and all (about 30) species studied have achiasmatic male meiosis (Kuznetsova et al., 2004, 2007; Grozeva et al., 2004). The subfamily Micronectinae consist of four genera: *Micronecta* from Old World, *Synaptonecta* from SE Asia and Florida, *Monogobia* and *Tenagobia* from Tropical and Neotropical America. In the genus *Tenagobia*, the chromosome formula of *T. (Fuscagobia) fuscata* (Corixoidea, Micronectidae) is reported to be  $2n=30(28+XY)$ , and male meiosis is achiasmatic (Ituarte, Papeschi, 2004). In the genus *Micronecta*, the chromosome formula  $2n=22+XY$  was reported by Southwood, Leston (1959) for *M. poweri*. This chromosome number was confirmed in the present study. It was also shown that *M. griseola* and *M. scholtzi* shared the same chromosome number.

Ituarte, Papeschi (2004) were first to find achiasmatic spermatogenesis in the family Micronectidae in the genus *Tenagobia*. The suggestion of Bachmann (1981) that the genera *Tenagobia* and *Micronecta* have separated very early in the evolution and the lack of any previous information of the presence of achiasmatic meiosis in *Micronecta* species on the other, led Ituarte, Papeschi (2004) to suggest that meiosis without chiasmata is restricted to the genus *Tenagobia* in the family and could be related to the specialized biology of the species in this genus. This suggestion got no support in the present study. Merely, our finding shows that achiasmatic meiosis originated before the genera *Micronecta* and *Tenagobia* separated within the family Micronectidae, but after the separation of the families Micronectidae, Corixidae, as in the latter chiasma forma-

tion in male meiosis is a rule (Waller, Angus, 2005; Bressa, Papeschi, 2007). In addition, the karyotypes of species in the family Corixidae are characterized by the presence of m-chromosomes (for a review see Bressa, Papeschi, 2007) while no m-chromosomes are found in species in the family Micronectidae (Ituarte, Papeschi, 2004; present study). The distinct cytogenetic features favour the suggestion of Nieser (2002) to consider the Micronectidae as a separate family instead of a subfamily of the family Corixidae.

As far as the findings of achiasmatic male meiosis was restricted to the infraorders Cimicomorpha and Leptomomorpha it was reasonable to suggest monophyletic origin of this type of meiosis in Heteroptera (Grozeva, Nokkala, 2002). However, findings showing it as a characteristic also for the family Micronectidae in Nepomorpha favour the idea that meiosis of this type has emerged more than once during the evolution (Ituarte, Papeschi, 2004). Whether it has developed twice or even more often remains to be elucidated in future studies.

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