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Chiasmate male meiosis in six species of water bugs from infraorders Nepomorpha and Gerromorpha (Insecta: Heteroptera)

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Abstract. The type of male meiosis is a stable character at the family level in the order Heteroptera and provides additional information on the relationships between taxa. The most common pattern, probably ancestral in the order is chiasmate meiosis; however achiasmate meiosis has been described in five families of terrestrial Heteroptera, all belonging to the infraorder Cimicomorpha (Anthocoridae, Microphysidae, Cimicidae, Miridae, Nabidae). Among water bugs, achiasmate meiosis is reported in the families Saldidae (Leptopodomorpha) and Micronectidae (Nepomorpha). Regarding the third infraorder of water bugs, Gerromorpha, data on meiotic patterns are absent, except for the *Limnogonus aduncus* Drake, Harris, 1933 (Gerridae) possessing chiasmate meiosis. In this paper, the male meiotic pattern of six water bugs species from infraorders Nepomorpha (*Plea minutissima minnutissima* Leach, 1817) and Gerromorpha (*Mesovelia furcata* Mulsant, Ray, 1852, *Microvelia reticulata* (Burmeister, 1835), *Gerris costae fieberi* Stichel, 1938, *Hydrometra gracilenta* Horváth, 1899, *Velia pelagonensis* Hoberlandt, 1941) is studied, and the karyotypes of the last two species are described for the first time. In the species examined, bivalents are chiasmate, so all these species possess chiasmate meiosis in males.

Key words: water Heteroptera, holokinetic chromosomes, chiasmate/achiasmate male meiosis.

Introduction

One of the unique cytogenetic characters of the true bugs (the order Heteroptera) along with the holokinetic chromosomes is the type of male meiosis, which is chiasmate in some while achiasmate in other groups. The meiotic pattern is stable at the family level and provides additional information on the relationships between higher taxa. The most common pattern, probably ancestral for the Heteroptera, is the chiasmate type. However, achiasmate meiosis has been found in five families of terrestrial Heteroptera, belonging to the infraorder Cimicomorpha (Anthocoridae, Microphysidae, Cimicidae, Miridae, Nabidae) (Nokkala, Nokkala, 1984, 1986a, b; Nokkala, Grozeva, 2000; Grozeva, Nokkala, 2002; Kuznetsova et al., 2007). Several hundred species studied in other terrestrial infraorders, Dipsocoromorpha and Pentatomomorpha, have been shown to possess chiasmate meiosis in males (Ueshima, 1979; Grozeva, Nokkala 1996). Among the water (heteropteran) bugs achiasmate meiosis has been (earlier) described in the infraorder

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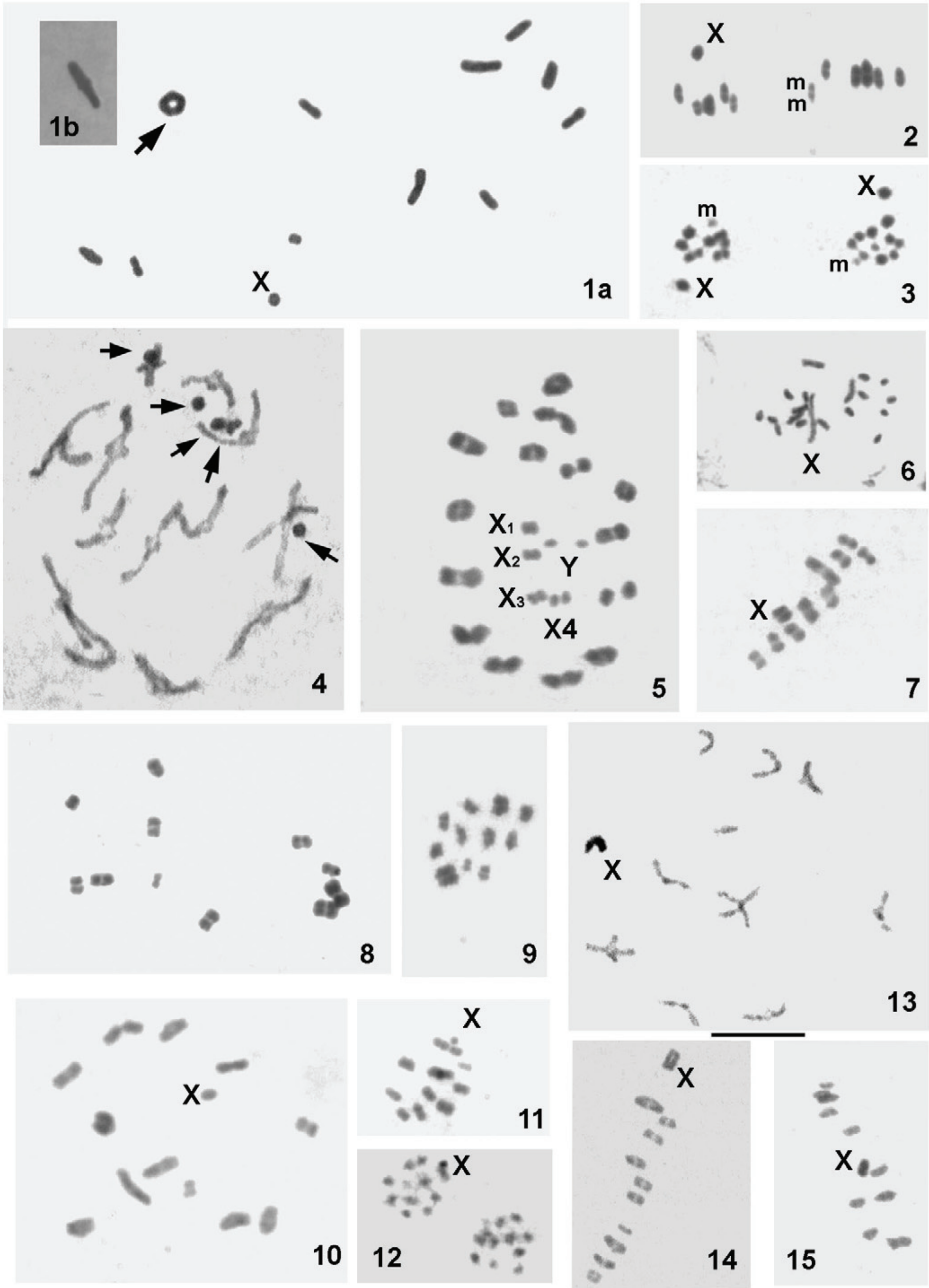

Figs 1-15. Male meiotic karyotypes of five species of Heteroptera. **1-3.** *Plea minutissima minutissima*, 2n=20+2m+X. **1**, **a, b** - prometaphase, the largest bivalent with two (**a**) or one (**b**) chiasmata. **2** - metaphase I. **3** - metaphase II. **4-5 -** *Mesovelia furcata*, 2n=30+$X_1X_2X_3X_4$Y. **4** - diakinensis. **5** - metaphase I. **6-7 -** *Hydrometra gracilenta*, 2n=18+X. **6** – spermatogonial metaphase. **7** – metaphase I. **8-9.** *Microvelia reticulata*, 2n= 20+X. **8** - metaphase I. **9** - metaphase II. **10-12 -** *Velia* (*Plesiovelia*) *pelagonensis*, 2n=24+X. **10** - prometaphase I. **11** - metaphase I. **12** - telophase II. **13-15 -** *Gerris costae fieberi*, 2n=20+X. **13** - diakinesis. **14** - metaphase I. **15** - metaphase II. Bar = 10 µm.

Table. Material used for chromosome analysis.

№	Family/Species	Place and date of collection	Number of specimens studied
1.	**Pleidae** *Plea minutissima minutissima* Leach, 1817, 2n=20+2m+X0	Bulgaria, Schkorpilovtsi, Fandaklijska river, 6.07.2008	1♂
2.	**Mesoveliidae** *Mesovelia furcata* Mulsant et Ray, 1852	Bulgaria, Schkorpilovtsi, Fandaklijska river, 6.07.2008	14♂
3.	**Hydrometridae** *Hydrometra gracilenta* Horváth, 1899	Finland, vicinity of Turku, July 2008	5♂
4.	**Veliidae (Microveliinae)** *Microvelia reticulata* (Burmeister, 1835)	Finland, vicinity of Turku, July 2008	18♂, 4 larvae
5.	**Veliidae (Veliinae)** *Velia* (*Plesiovelia*) *pelagonensis* Hoberlandt, 1941	Bulgaria, Slavianka Mt., Petrovska river, 550m, 2.07.2008	11♂
6.	**Gerridae** *Gerris costae fieberi* Stichel, 1938	Bulgaria, Osogovo Mt., in a pool under Ruen peak, 1600m, 22.06.2008	3♂

Leptopodomorpha (Saldidae) (Nokkala, Nokkala, 1983), and recently it has also been reported in the infraorder Nepomorpha in the family Micronectidae (Ituarte, Papeschi, 2004; Grozeva et al., 2008). In the infraorder Gerromorpha there are no observations available on species with achiasmate meiosis.

In this paper, the male meiotic pattern of six water bug species from the infraorders Nepomorpha (*Plea minutissima minnutissima* Leach, 1817) and Gerromorpha (*Mesovelia furcata* Mulsant, Ray, 1852, *Microvelia reticulata* (Burmeister, 1835), *Gerris costae fieberi* Stichel, 1938, *Hydrometra gracilenta* Horváth, 1899, *Velia pelagonensis* Hoberlandt, 1941) is studied, and the karyotypes of the last two species are described for the first time. Bivalents in spermatogenesis of all species examined are chiasmate.

Material and methods

The data on the insects used in the present study are presented in the Table. The insects were fixed alive in 3:1 fixative (96% ethanol - glacial acetic acid mixture). The abdomen was dissected in 45% acetic acid and the internal reproductive system was analyzed under a stereomicroscope. The gonads were squashed in a small drop of 45% acetic acid. The cover slips were removed by a dry ice technique. Slides were dehydrated in fresh fixative (3:1) and air dried. To study the number and the behavior of the chromosomes the preparations were stained by Schiff-Giemsa method after Grozeva, Nokkala (1996). Chromosome spreads were analyzed using a Laborlux 12 (Leitz, Wetzlar, Germany) and Olympus BX 51 microscopes and documented by digital photo camera Moticam 2000. The preparations are preserved in the collection of the Laboratory "Cytotaxonomy and evolution", Institute of Zoology BAS, Sofia.

Results

Nepomorpha

Pleidae

Plea minutissima minutissima Leach, 1817, 2n=20+2m+X.

In the present study, the chromosome formula reported earlier for this species by Jande (1959, 1961) was confirmed. At prometaphase plates (PMI), most bivalents display one terminal chiasma, while the largest bivalent more often has two terminal chiasmata resulting in a ring (Fig. 1, a), or one subterminal chiasma (Fig. 1, b). At metaphase I (MI), 10 autosomal bivalents gradually decreasing in size, a pair of comparatively large m-chromosomes and the univalent X chromosome as large as the largest autosome can be seen (Fig. 2). The first division is reductional for the autosomes, whereas the sex chromosome undergoes equational separation (post-reduction). As a result, at metaphase II (MII) daughter cells each contain 12 elements (10 autosomes, m-chromosome and the daughter X chromosome) (Fig. 3).

Gerromorpha

Mesoveliidae

Mesovelia furcata Mulsant, Ray, 1852, $2n=30+X_1X_2X_3X_4Y$.

In this species, the testes were examined. Every testis consists of one elliptic orange follicle. Our study confirmed the chromosome formula of this species published by Ekbolm (1941). At diakinensis, 15 bivalents each with one terminal or subterminal chiasma and five univalent sex chromosomes can be seen (Fig. 4). At MI, four X and one Y sex chromosomes are placed inside the circle formed by the autosomal bivalents (Fig. 5).

Hydrometridae

Hydrometra gracilenta Horváth, 1899, 2n=18+X.

The karyotype of this species is described for the first time. In Fig. 6, a spermatogonial metaphase displays 18 autosomes and the very large X chromosome, which is the largest chromosome of the set. The autosomes gradually decrease in size. In MI, there are 9 bivalents with terminal chiasmata and a univalent X chromosome (Fig. 7). The chromatids of the X lie in parallel to the equator and are oriented to different poles

Veliidae

Microvelia reticulata (Burmeister, 1835), 2n=20+X.

Cobben (1968) interpreted the karyotype of *M. reticulata* as 21 (XO). In the present study we confirmed this chromosome formula, as both MI and MII (Figs 8 and 9) show 11 chromosome elements. It is not possible to identify the X chromosome reliably. At MI, all bivalents displayed terminal chiasmata (Fig. 8).

Velia (*Plesiovelia*) *pelagonensis* Hoberlandt, 1941, 2n=24+X.

The karyotype of this species is studied for the first time. Fig. 10 shows prometaphase I with 12 bivalents, every bivalent possessing a terminal or subterminal chiasma. At MI (Fig. 11), the autosomal bivalents gradually decrease in size, and the X chromosome is one of the smallest in the set. The first division, reductional for the autosomes but equational for the X chromosome, results in two kinds of TII cells: one with 12 autosomes and the daughter X, and the other with 12 autosomes only (Fig. 12).

Gerridae

Gerris costae fieberi Stichel, 1938, 2n=20+X.

Our observations confirm the chromosome formula 2n=20+X earlier reported for this species by Southwood and Leston (1959). At diakinesis (Fig. 13), autosomal bivalents with terminal or interstitial chiasmata and the X chromosome can be seen. At MI (Fig. 14) and MII (Fig. 15), the X chromosome is easily recognized because it is heteropycnotic and one of the largest chromosomes in the set. Fig. 14 shows a MI plate, in which the chromatids of the X lie in parallel to the equator and are oriented to different poles.

Discussion

As was mentioned in Introduction, the most common pattern, probably ancestral in the order Heteroptera, is chiasmate male meiosis (Nokkala et al., 2002). Among the terrestrial true bugs there are no data available on karyotypes and meiosis in the infraorder Enicocephalomorpha, while in the infraorders of Dipsocoromorpha and Pentatomomorpha several hundred species studied so far were shown to display chiasmate meiosis in males (Ueshima, 1979; Grozeva, Kuznetsova, 1993; Grozeva, Nokkala, 1996). Within the large infraorder Cimicomorpha, the families Tingidae (Grozeva, Nokkala 2001) and Reduviidae (Severi-Aguiar et al, 2006) display the chiasmate meiotic pattern, while in the Anthocoridae, Microphysidae, Cimicidae, Miridae, Nabidae only achiasmate meiosis has been described (see Grozeva et al., 2008).

Although there are no reviews dedicated to the type of meiosis in the water bugs (Nepomorpha, Gerromorpha, Leptopodomorpha), it can be supposed that the species with chiasmate meiosis also predominate in these groups. In two *Saldula* Van Duzee, 1914 species (Saldidae) achiasmate meiosis has been earlier reported (Nokkala, Nokkala, 1983), but there are no data on the meiosis of the three other families of the infraorder Leptopodomorpha. Recently, achiasmate meiosis has been reported for the first time in the infraorder Nepomorpha. In this infraorder, species of the family Corixidae display chiasmata during the male meiosis (Waller, Angus, 2005), but four species of the family Micronectidae (*Tenagobia* (*Fuscagobia*) *fuscata* (Stål, 1859), *Micronecta scholtzi* (Fieber, 1860), *M. poweri* (Douglas et Scott, 1869) and *M. griseola* Horvath, 1899) were found to have achiasmate male meiosis (Ituarte, Papeschi, 2004; Grozeva et al., 2008), These data elucidate the taxonomic problems within the Corixoidea, and provide additional support for the idea by Nieser (2002) to consider Micronectidae separate from the family Corixidae.

Recently, in the infraorder Gerromorpha terminal and interstitial chiasmata were reported for two *Hebrus* Curtis, 1833 species (Hebridae) (Nokkala, Nokkala, 1999), as well as for *Limnogonus aduncus* Drake, Harris, 1933 (Gerridae) (Castanhole et al., 2008). However, for the other over twenty species examined only the number of chromosomes is given (see Ueshima, 1979) without any information about the type of meiosis in males.

In the present study, chiasmata were described in six water bugs species, belonging to one family of the Nepomorpha (Pleidae) and four families of the Gerromorpha (Hydrometridae, Veliidae, Mesoveliidae, Gerridae). These observations confirm the suggestion that chiasmate male meiosis is the most common and probably ancestral meiotic pattern in the order Heteroptera, well distributed among both the terrestrial and the water bugs. Additional studies of different heteropteran taxa, and especially of water bugs, are necessary to learn more about the distribution of the meiotic patterns among the true bugs as a whole.

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