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Meiotic karyotypes and testis structure of 14 species of the planthopper tribe Issini (Hemiptera: Fulgoroidea, Issidae)

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Abstract. Karyotypes and testis structure of 14 species representing 9 genera (*Latissus*, *Bubastia*, *Falcidius*, *Kervillea*, *Mulsantereum*, *Mycterodus*, *Scorlupaster*, *Scorlupella* and *Zopherisca*) of the planthopper tribe Issini (Issidae) are presented. All the karyotypes are illustrated by meiotic and occasionally mitotic figures. The male karyotypes of most of the species analyzed are 2n = 26 + X, the exception being *Falcidius limbatus*, which has a karyotype of 2n = 24 + neo-XY. The latter is the first report of the neo-XY system in the family Issidae. The species studied are found to be similar in having NORs on the largest pair of autosomes, but differ significantly in the amount and distribution of C-heterochromatin along the chromosomes. In contrast to the conserved chromosome numbers, the highly variable follicle number in the testes suggests rapid evolution of the tribe Issini. On the basis of its specific follicle number, it is proposed that *Zopherisca tendinosa skaloula* Gnezdilov & Drosopoulos, 2006 be upgrade to a species: *Z. skaloula* stat. n. The cytological and taxonomic significance of results presented are discussed.

INTRODUCTION

The large cosmopolitan group of plant-sucking bugs, known as planthoppers, (the superfamily Fulgoroidea = the infraorder Fulgoromorpha) contains more than 9000 described species in 21 currently recognized extant families (Szwedo, 2007). The Issidae Spinola, 1939 is one of the largest and most diverse planthopper families comprising approximately 1000 described species and is considered as the closest group to the base of the monophyletic so called "higher" Fulgoroidea (Emeljanov, 1990; Shcherbakov, 2006). According to recent revisions (Gnezdilov, 2003a; 2009), the Issidae sensu stricto contains a single subfamily with four tribes: Issini Spinola, 1839 (= Thioniini Melichar, 1906), Parahiraciini Cheng & Yang, 1991, Hemisphaeriini Melichar, 1906 and Colpopterini Gnezdilov, 2003. The tribe Issini is the largest and is classified into three subtribes: Issina Spinola, 1839, Hysteropterina Melichar, 1906 and Agalmatiina Gnezdilov, 2002 (Gnezdilov, 2002, 2003b).

The karyotypes of the Issidae were the subject of study in 1962, when Halkka (1962) recorded 2n = 26 + X for males of *Colpoptera* sp. (Colpopterini). The currently available evidence on karyotypes reviewed by Maryańska-Nadachowska et al. (2006) is for 22 species (14 genera, 3 subtribes) of the tribe Issini and one species (*Colpoptera* sp.) of the tribe Colpopterini. All Auchenorryncha have holokinetic chromosomes, in which the kinetic activity is spread over the entire length of the chromosome (Halkka, 1959). It is generally assumed that this character facilitates karyotype evolution by means of fragmentation or fusion of chromosomes (White, 1973). However among the auchenorhynchan groups that have been studied cytogenetically, the Issidae seems to be an example of apparent karyotype conservation with all species having 2n = 26 + X in males other than those of *Brahmaloca* sp. and *Latilica maculipes* (Melichar, 1906), which both have 2n = 24 + X.

Almost all the published studies on Issidae karyotypes have used a standard chromosome technique, which provides information mainly about number and size of chromosomes, and sex determining mechanisms with little or no other information on chromosome complements. The only exception is the recent investigation of *Hysteropterum albaceticum* Dlabola, 1983 and *Agalmatium bilobum* (Fieber, 1877) using a number of cytogenetic techniques with the aim of identifying cytogenetic markers and distinguishing between species with the modal karyotype of 2n = 26 + X and no detectable interspecies differences in karyotype structure. This study revealed considerable differences between these species in the amount of C-heterochromatin, its pattern of distribution along the chromosomes and stainability with the base specific fluorochromes DAPI and CMA_3 (Kuznetsova et al., 2009b).

In the present study, the chromosome complements and some details of male meiosis in fourteen issid species, all from the tribe Issini, using C-banding, fluorochrome DAPI-banding and silver nitrate banding, were analyzed. As a result, the distribution and nucleotide sequences of C-heterochromatin regions and the location of nucleolus organizing regions (NORs) in the karyotypes studied are reported here. The revealed banding and NOR patterns provided new markers for comparative cytogenetic characterization of species in the family Issidae. In addition, for each species, the structure of testes in terms of the

number of seminal follicles is also described. In a previous study (Maryańska-Nadachowska et al., 2006), it is shown that this character, unlike chromosome number, is highly variable in the Issidae, with however an apparent mode of 10 follicles per testis.

The new results accord well with earlier conclusions that there is a marked variability in follicle number and uniformity in chromosome number in the Issidae, at least in the tribe Issini, to which the overwhelming majority of hitherto studied species belong. The X0 male sex chromosome system is confirmed as characteristic of this group. Theoretically, this fact implies it is possible to evolve a derived sex chromosome system, in which the univalent X chromosome is translocated to an autosome giving rise to a neo-XY system. In support of this hypothesis is the first finding of a neo-XY issid species reported here.

MATERIAL AND METHODS

Insects

The localities from which the species were collected and the numbers of males analyzed are presented in Table 1. Freshly caught specimens were fixed in 3 : 1 ethanol/acetic acid.

Chromosome preparations

Chromosome spreads were obtained from testes as previously described (Maryańska-Nadachowska et al., 2006; Kuznetsova et al., 2009b). Testes were extracted from abdomens in a drop of 45% acetic acid; testicular follicles were separated and counted. Chromosome preparations were made by the squash method: testis material was squashed under a coverslip in a drop of 45% acetic acid; the coverslip was removed after freezing with dry ice, the preparation was dehydrated in freshly prepared 3 : 1 ethanol/acetic acid for 20 min and air-dried. The preparations were first observed under a phase contrast microscope at 400 ×. The best chromosome spreads were stained in several different ways.

Standard staining

For standard staining the method of Grozeva & Nokkala (1996) was used. The preparations were first subjected to hydrolysis in 1 N HCl at 60°C for 7 min and stained in Schiff's reagent for 20 min. After rinsing thoroughly in distilled water, the preparations were additionally stained in 4% Giemsa in Sorensen's buffer, pH 6.8 for 20 min, rinsed with distilled water, air-dried, and mounted in Entellan.

C-banding

For C-banding the method of Sumner (1972) with minor modifications was used. C-banding treatment was carried out using 0.2 N HCl at room temperature for 30 min, followed by 7–8 min treatment in saturated $Ba(OH)_2$ at room temperature and then incubated in 2 × SSC at 60°C for 1 h. After this, the preparations were finally stained in 4% Giemsa diluted in Sorensen's buffer for 10–15 min, then the excess stain removed by briefly rinsing in tap water before mounting in Entellan.

AgNOR-staining

For AgNOR-staining (silver nitrate impregnation) the 1-step method with colloidal developer of Howell & Black (1980) was used. Slides were incubated for 6–9 min at 60°C with 60 µl of $AgNO_3$ (0.5 g/ml) and 30 µl of a 2% gelatin containing 1% formic acid solution. The preparations were rinsed with distilled water and air-dried.

DAPI-banding

For fluorochrome application, the method of Donlon & Magenis (1983) was used with minor modifications. Some pretreated preparations for C-banding were stained with DAPI (4',6-diamidino-2-phenylindole; 0.4 µg/ml) for 5 min. To improve the fluorochrome staining, 0.5% methanol was included in the staining solutions (Kuznetsova et al., 2001). After staining, the preparations were rinsed in the McIlvaine buffer, pH 7 and mounted in an anti-fade medium (700 µl of glycerol, 300 µl of 10 mM McIlvaine buffer, pH 7, and 10 mg of N-propyl gallate).

The preparations were studied under a Leica MM 4000 microscope at a magnification of 1000 × or (the fluorochrome-stained

TABLE 1. Collection locality, number of males studied, diploid chromosome number and number of follicles per testis in fourteen species of the tribe Issini (Issidae).

Taxa	Collection locality	No. of males studied	2n♂	Number of follicles per testis
Issidae Spinola				
Issinae Spinola				
Issini Spinola				
Issina Spinola				
Latissus dilatatus (Fourcroy, 1785)	Italy	5	26 + X	12
Hysteropterina Melichar				
Bubastia (*Bubastia*) *obsoleta* (Fieber, 1877)	Greece	4	26 + X	10
B. (*B.*) *saskia* Dlabola, 1984	Greece	4	26 + X	10
Falcidius doriae (Ferrari, 1884)	Italy	3	26 + X	10
F. limbatus (A. Costa, 1864)	Italy	4	24 + XY	no data
Kervillea (*Kervillea*) *basiniger* (Dlabola, 1982)	Greece	2	26 + X	10
K. (*Corymbius*) *tekirdagica* (Dlabola, 1982)	Greece	2	26 + X	10
Mulsantereum abruzicum (Dlabola, 1983)	Southern Italy	2	26 + X	10
Mycterodus (*Semirodus*) *colossicus* (Dlabola, 1987)	Greece	3	26 + X	18
M. (*Mycterodus*) *etruscus* Dlabola, 1980	Italy	1	26 + X	16
M. (*M.*) *intricatus* Stål, 1861	Crimea	1	26 + X	20
Scorlupaster asiaticum (Lethierry, 1878)	Kazakhstan	2	26 + X	9
Scorlupella discolor (Germar, 1821)	Crimea	1	26 + X	6
Zopherisca tendinosa tendinosa (Spinola, 1839)	Greece	3	26 + X	28

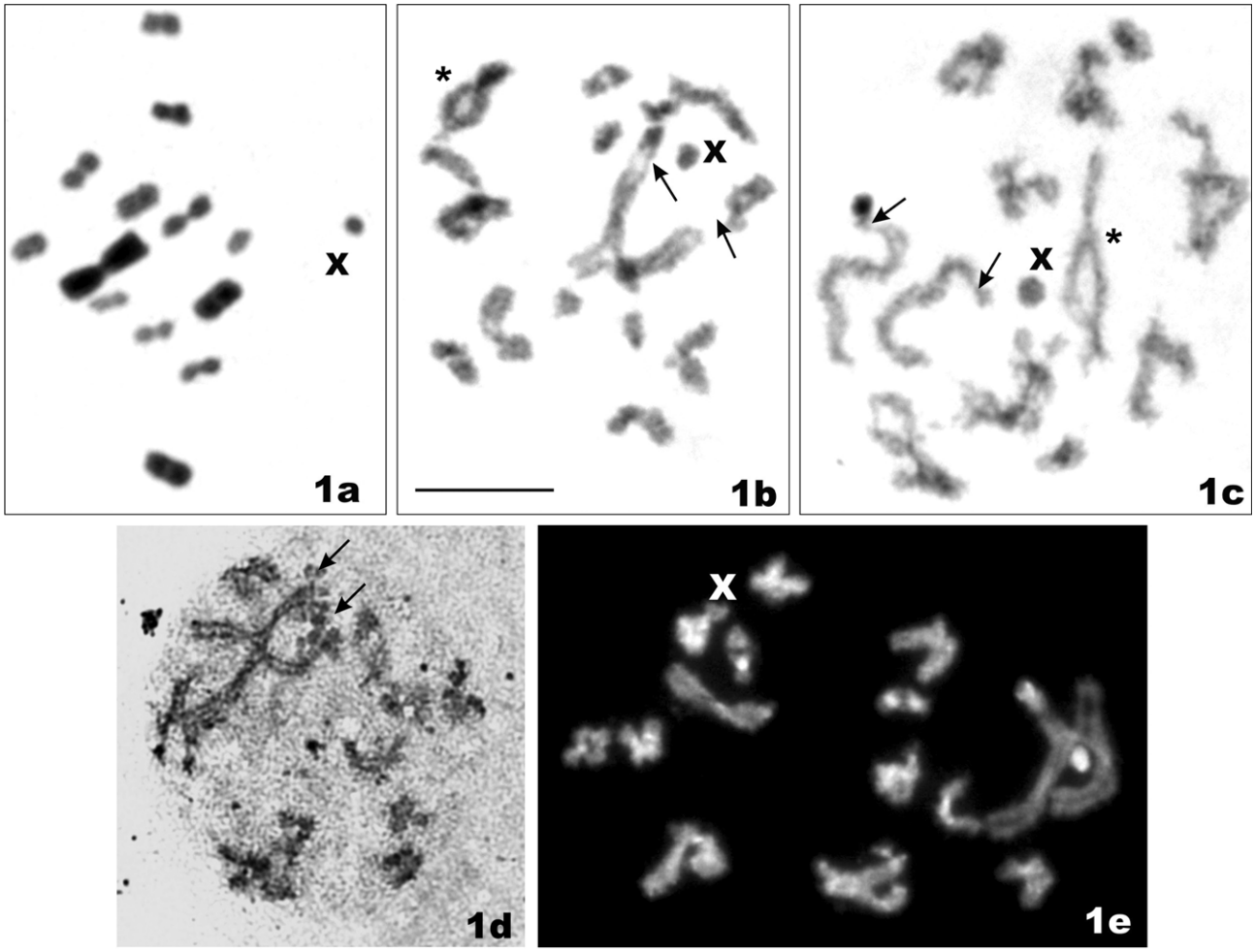

Fig. 1. Meiotic chromosomes of *Latissus dilatatus*: a – metaphase I, n = 13 + X; b–e – C-banded (b, c), silver stained (d) and DAPI-banded (e) diplotene. Arrows indicate gaps (b, c) and sites of NOR (d) on the largest bivalent; asterisks indicate bivalents with two chiasmata (b, c). Bar indicates 10 μm.

preparations) a Dialux 22 fluorescence microscope at 1000 ×. The micrographs were taken using a Nikon DS-U1 CCD camera.

A total of 42 adult males were studied in terms of meiotic karyotypes, different stages of meiosis and the number of testicular follicles. Except for *Mycterodus intricatus*, none of the males showed spermatogonial mitoses. The number of specimens investigated ranged from one (for one species) to five per species (Table 1). Male diploid chromosome complements and the number of testicular follicles per testis of the species studied are presented in the Table 1.

RESULTS

Latissus Dlabola, 1974

Published data. Absent.

L. dilatatus (Fourcroy, 1785): 2n = 26 + X (Fig. 1a–e).

In males of this species the testes each consist of 12 follicles. During meiotic stages, 13 autosomal bivalents and an X chromosome univalent were observed; one of the bivalents was noticeably larger than the others; the remaining bivalents were of gradually decreasing size; and the X chromosome appeared roundish in form and close in size to the medium-sized half-bivalents (Fig. 1a–e). At metaphase I (MI) the X chromosome is positioned outside the plate formed by autosome bivalents (Fig. 1a) as is usual in Auchenorrhyncha (Halkka, 1959; Kuznetsova, 1985; Kuznetsova et al., 1998, 2009a, b; Maryańska-Nadachowska et al., 2006). At diplotene, the largest bivalent had a gap in the subtelomeric region of each homologue (Fig. 1b, c). Silver staining revealed that these regions are argyrophilic, which suggests that they are NORs (Fig. 1d) and the largest bivalent is hence the NOR-bivalent. In one specimen the homologues of the largest bivalent appeared as univalents without any physical connections between them (Fig. 1 c). In every bivalent there was only one terminal or interstitial chiasma, except for the larger bivalents that sometimes showed two crossover points (Fig. 1b, c). Fig. 1e shows a diplotene nucleus stained with DAPI after C-banding treatment (without Giemsa staining). All the bivalents, but not the X chromosome, show AT-rich/DAPI positive blocks of various brightness and location (either terminal or interstitial).

Bubastia Emeljanov, 1975

Published data. *B.* (*Bubastia*) *taurica* (Kusnezov, 1926): 2n = 26 + X; 10 fol./testis (Maryańska-Nadachowska et al., 2006).

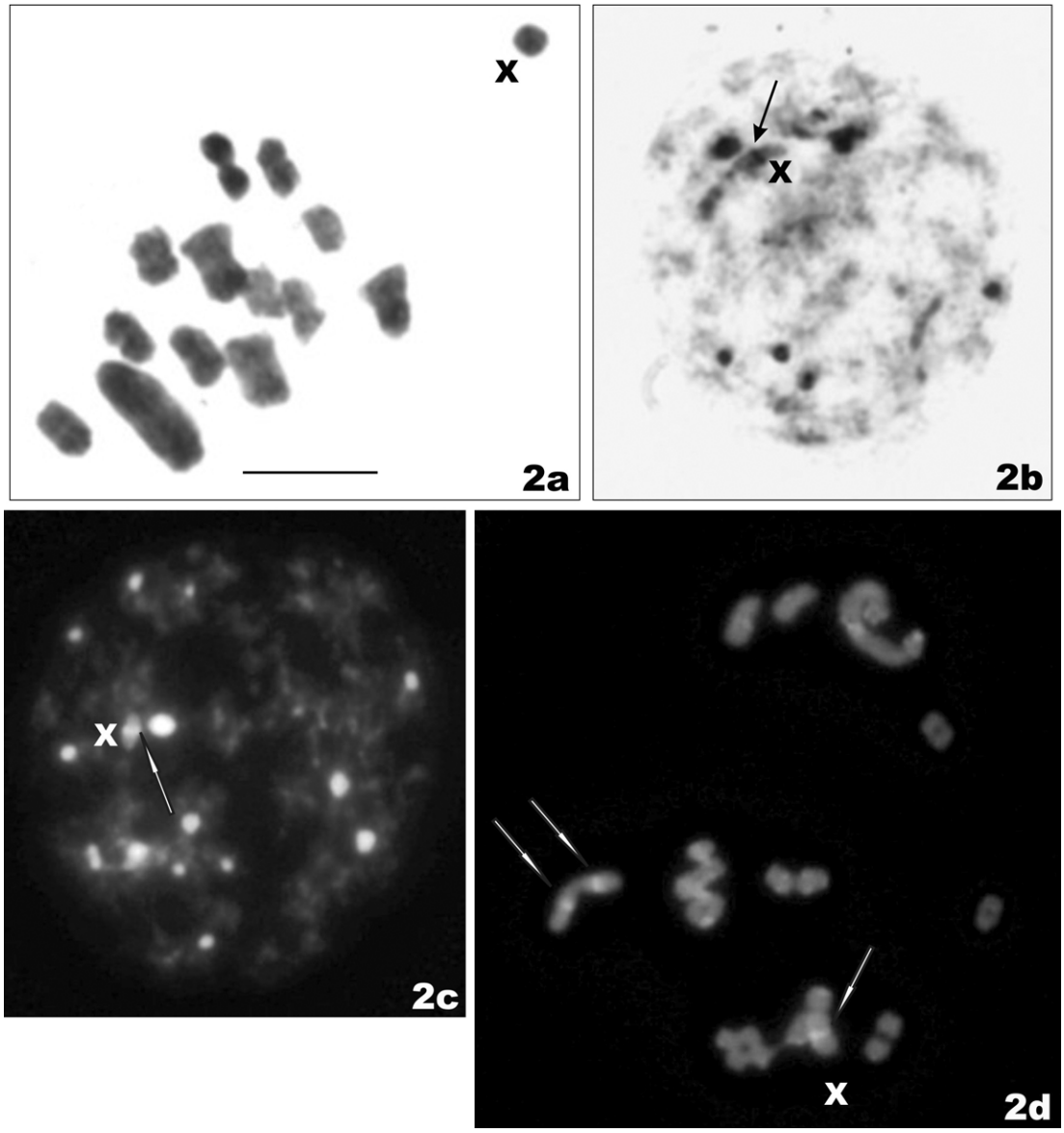

Fig. 2. Meiotic chromosomes of *Bubastia obsoleta*: a – metaphase I, n = 13 + X; b, c – C-banded (b) and DAPI-banded (c) premeiotic interphase; d – DAPI-banded diakinesis. Arrows indicate C-blocks and DAPI-positive bands on the X chromosome (b–d) and a marker bivalent with two interstitial bands (d). Bar indicates 10 μm.

B. (*Bubastia*) *obsoleta* (Fieber, 1877): 2n = 26 + X (Fig. 2a–d).

Testes each consist of 10 follicles. At spermatocyte MI there were 13 bivalents and an X chromosome univalent; one of the bivalents was very large, whereas the other bivalents gradually decreased in size. At this stage, the X chromosome appeared to be round in form and close in size to the medium-sized half-bivalents; it was invariably located far from the autosomes (Fig. 2a). Heterochromatin dots were found scattered in premeiotic interphase cells after conventional C-banding (Fig. 2b). DAPI-banding produced at least 14 bright AT-rich signals of various size and brightness in the interphase nuclei (Fig. 2c) suggesting there is a large amount of C-heterochromatin in this species. The X chromosome is easily recognized in every interphase cell due to its heteropycnotic state and the presence of a bright interstitial block. At diplotene/diakinesis there were AT-rich/DAPI-positive blocks mainly in the telomeric regions of the majority of the bivalents, however bright interstitial signals were present in every homologue of one of the larger bivalents (probably the second in size) and the X chromosome (Fig. 2d).

B. (*B.*) *saskia* Dlabola, 1984: 2n = 26 + X (Fig. 3a–c).

Both testis structure and karyotype are similar to that in *B. obsoleta.* Testes each consist of 10 follicles. During meiotic stages, 13 autosomal bivalents and an X chromosome univalent were observed; one of the bivalents was noticeably larger than the others, whereas the remaining bivalents gradually decreased in size, and the X chromosome was larger (Fig. 3a–c). At spermatocyte MI, the X chromosome was roundish in form and invariably located far from autosomes (Fig. 3a). At this stage, the actual size of the X chromosome was difficult to determine due to its

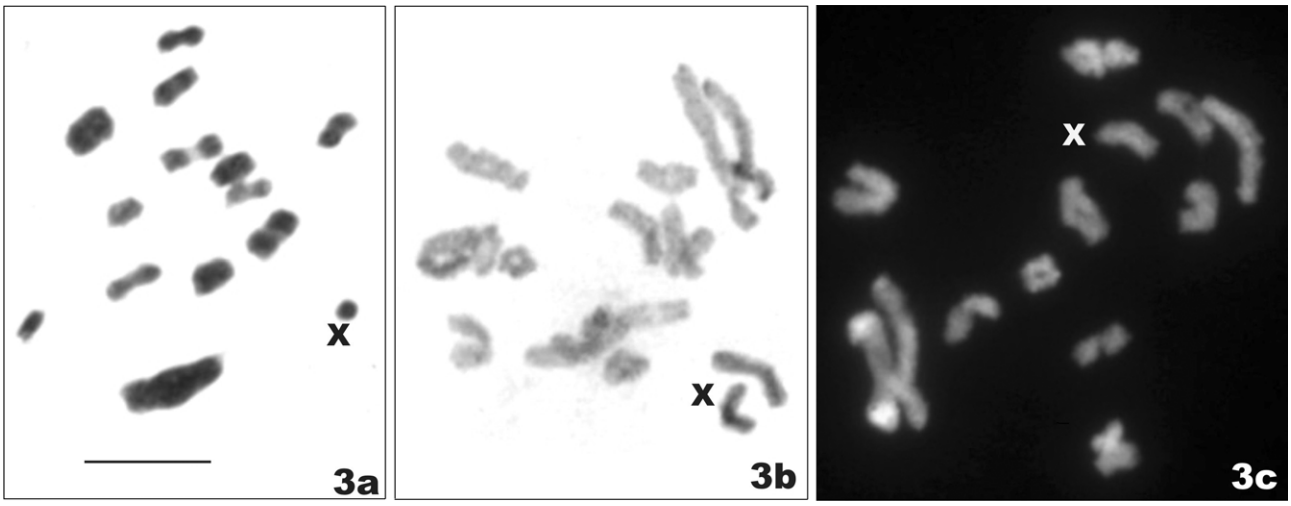

Fig. 3. Meiotic chromosomes of *Bubastia saskia*: a – metaphase I, n = 13 + X; b, c – C-banded (b) and DAPI-banded (c) diplotene/diakinesis. Bar indicates 10 μm.

positive heteropycnosis. However, during the prophase stages the X chromosome clearly appeared similar in size to the larger half-bivalents (Fig. 3b, c). There was usually a single chiasma on all the bivalents except the largest, which either usually had one chiasma at an interstitial position (Fig. 3b, c) or two chiasmata (not shown). C-banding revealed there is a small amount of C-heterochromatin in this karyotype, however the largest bivalent had several C-bands and the X chromosome one prominent interstitial C-block (Fig. 3b). When DAPI was used after C-banding treatment there is an area of bright fluorescence at one end of every homologue of the largest bivalent (Fig. 3c).

***Falcidius* Stål, 1866**

Published data. Absent.

F. doriae (Ferrari, 1884): 2n = 26 + X (Fig. 4a–e).

Testes each consist of 10 follicles. At diakinesis, there were 13 autosome bivalents, one of which was very large and the remaining bivalents constituted a series that decreased in size, and the double-stranded X chromosome (Fig. 4a; C-banding). The sex chromosome appeared similar in size to large-sized half-bivalents and was marked by a bright interstitial C-band. The chromosomes were found to contain a small amount of C-heterochromatin except for the X chromosome and the largest bivalent (Fig. 4a–e). The largest bivalent had both a telomere and interstitial C-bands. Some of these bands were not apparent in preparations in all the cells from one specimen, but one interstitial (subterminal) band was present in each homologue of the majority of cells (Fig. 4b–e).

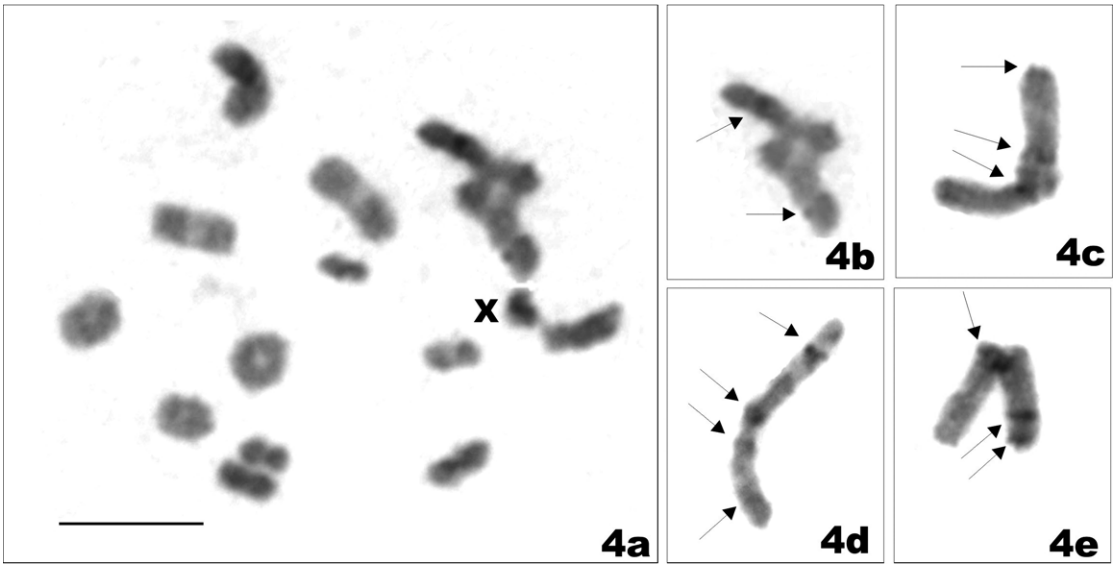

Fig. 4. Meiotic chromosomes of *Falcidius doriae*: a – C-banded diakinesis, n = 13 + X; b–e – C-banded largest bivalent. Arrows indicate C-blocks on the largest bivalent. Bar indicates 10 μm.

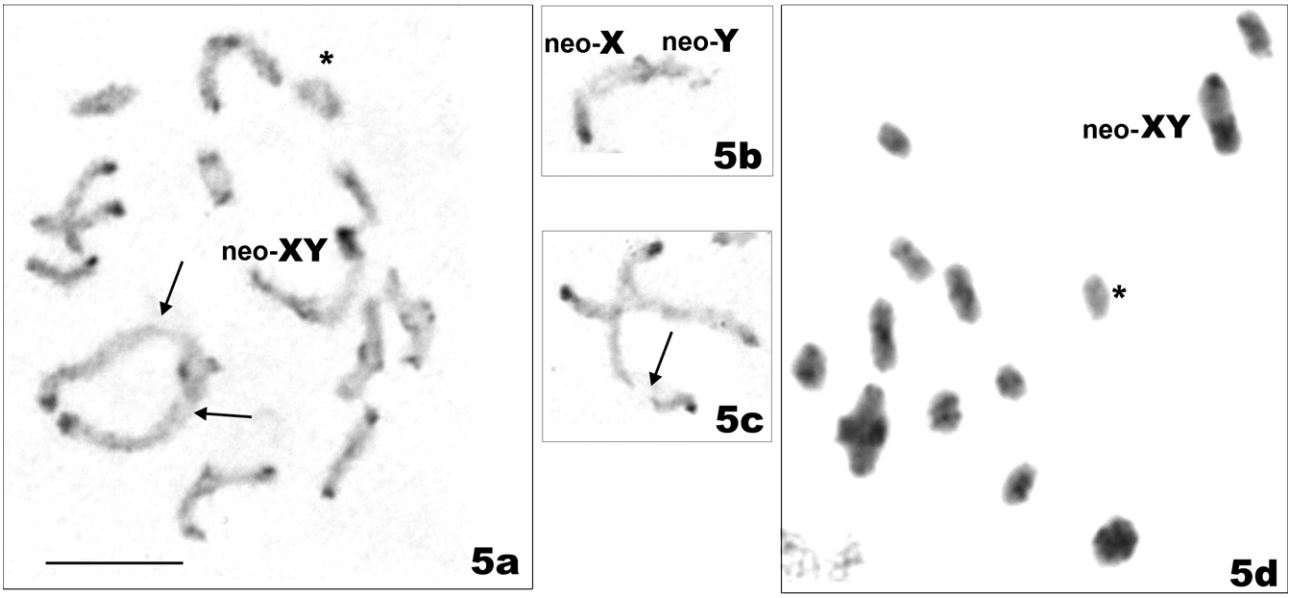

Fig. 5. Meiotic chromosomes of *Falcidius limbatus*: a, d – C-banded diplotene/diakinesis (a) and diakinesis/metaphase I (d), n = 12 + neo-XY; b – C-banded neo-XY bivalent; c – C-banded largest bivalent. Arrows indicate gaps on the largest bivalent (a, c); asterisks indicate a bivalent without C-blocks (a, d). Bar indicates 10 μm.

F. limbatus (A. Costa, 1864): 2n = 24 + neo-XY (Fig. 5a–d).

Testes were not studied. Conventionally C-banded cells at diplotene and diakinesis, and separate chromosomes are shown in Fig. 5a–d. Both stages show 13 bivalents, suggesting a male karyotype of 2n = 24 + XY. One of the bivalents stands out because it is very large. The sex bivalent is second in size and heteromorphic in form, with one fully euchromatic chromosome (most likely the Y) and a longer chromosome (probably the X). The latter has a euchromatic part similar in size to the Y and a short heterochromatic segment (Fig. 5a, b, d). The origin of this X chromosome is ascribed to a translocation between the original X chromosome and an autosome of a karyotype of 2n = 26 + X0 like that of *F. doriae.* Thus, the sex chromosomes of *F. limbatus* are referred to as neo-X and neo-Y, respectively. The largest autosomal bivalent has an interstitial gap in both homologues (Fig. 5a), or more often in only one (Fig. 5c) homologue. This gap is probably associated with a nucleolar organizer region (NOR); however Ag-staining did not confirm this. On all but one (marked by asterisk) of the bivalents there are C-heterochromatic bands (Fig. 5a, d). In autosomal bivalents, C-bands were located at the ends of the chromosomes, sometimes at both ends, but more often at one end or on one homologue only (Fig. 5a). There was one terminal or subterminal chiasma on every bivalent and the sex chromosome bivalent, except the largest bivalent on which there were sometimes two chiasmata (not shown).

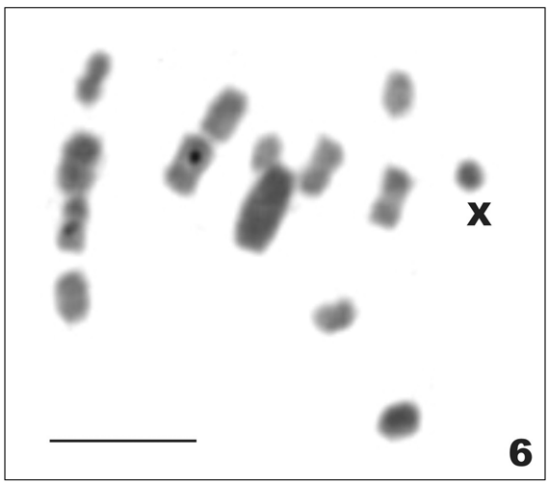

Fig. 6. Meiotic chromosomes of *Kervillea basinger*: diakinesis/metaphase I, n = 13 + X. Bar indicates 10 μm.

Kervillea **Bergevin, 1918**

Published data. Absent.

K. (*Kervillea*) *basiniger* (Dlabola, 1982): 2n = 26 + X (Fig. 6).

Testes each consist of 10 follicles. Fig. 6 shows a diakinesis/metaphase I transition with 13 autosome bivalents, one of which is noticeably larger than the others, and an X chromosome univalent, which is located at some distance from the bivalents.

K. (*Corymbius*) *tekirdagica* (Dlabola, 1982): 2n = 26 + X (Fig. 7).

This species shares with *K. basiniger* 10 follicles per testis and 2n = 26 + X in males. Fig. 7 shows a spermatocyte diakinesis/metaphase I transition with 13 autosome bivalents, one of which is very large, and a double-stranded X chromosome univalent. The latter is located among the bivalents but close to the outlying area of the nucleus. Each bivalent appears to have a single terminal chiasma, except for the largest, which has a subterminal chiasma.

***Mulsantereum* Gnezdilov, 2002**

Published data. Absent.

M. abruzicum (Dlabola, 1983): 2n = 26 + X (Fig. 8a–c).

Testes each consist of 10 follicles. Fig. 8 represents consecutive stages of meiosis. At both the diakinesis/metaphase I transition (Fig. 8a) and metaphase I (Fig. 8b) there are 13 autosomal bivalents, one of which is larger than the others, and an X chromosome univalent. The latter is located in an outlying area of the nucleus; it still appears among the bivalents at diakinesis (Fig. 8a) but far from the bivalents both at MI (Fig. 8b) and anaphase I (Fig. 8c). The bivalents each have a single chiasma and only the largest bivalent appears in the form a ring at diakinesis, which suggests the presence of two chiasmata (Fig. 8a).

***Mycterodus* Spinola, 1839**

Published data. *M. (Semirodus) pallens* (Stål, 1861): 2n = 26 + X; 18 fol./per testis (Maryańska-Nadachowska et al., 2006).

M. (*Semirodus*) *colossicus* (Dlabola, 1987): 2n = 26 + X (Fig. 9a–d).

Testes each consist of 18 follicles. At spermatocyte diakinesis/metaphase I there are 13 autosome bivalents, one of which is noticeably larger than the others, and an X chromosome univalent located at a distance from the bivalents (Fig. 9a). C-banded diakinesis/metaphase I transition stage indicate very large telomeric blocks on the largest pair of autosomes, small C-blocks on several bivalents and none on the X chromosome (Fig. 9b). After C-banding staining all C-heterochromatic regions showed DAPI-bright fluorescence (Fig. 9d) indicating they are AT-rich. There are no DAPI-positive bands on the X chromosome. Staining with silver nitrate revealed

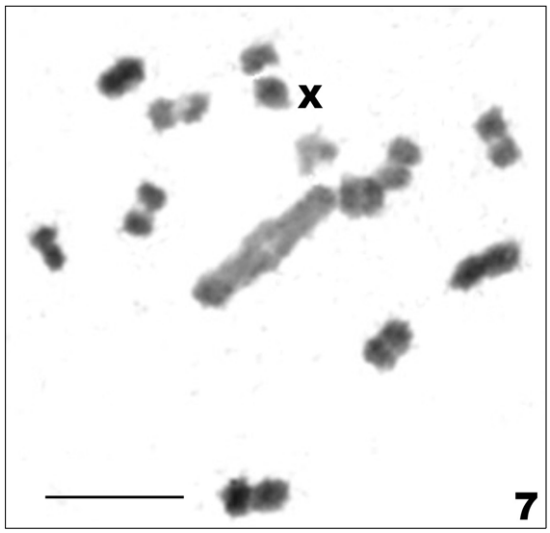

Fig. 7. Meiotic chromosomes of *Kervillea tekirdagica*: diakinesis/metaphase I, n = 13 + X. Bar indicates 10 μm.

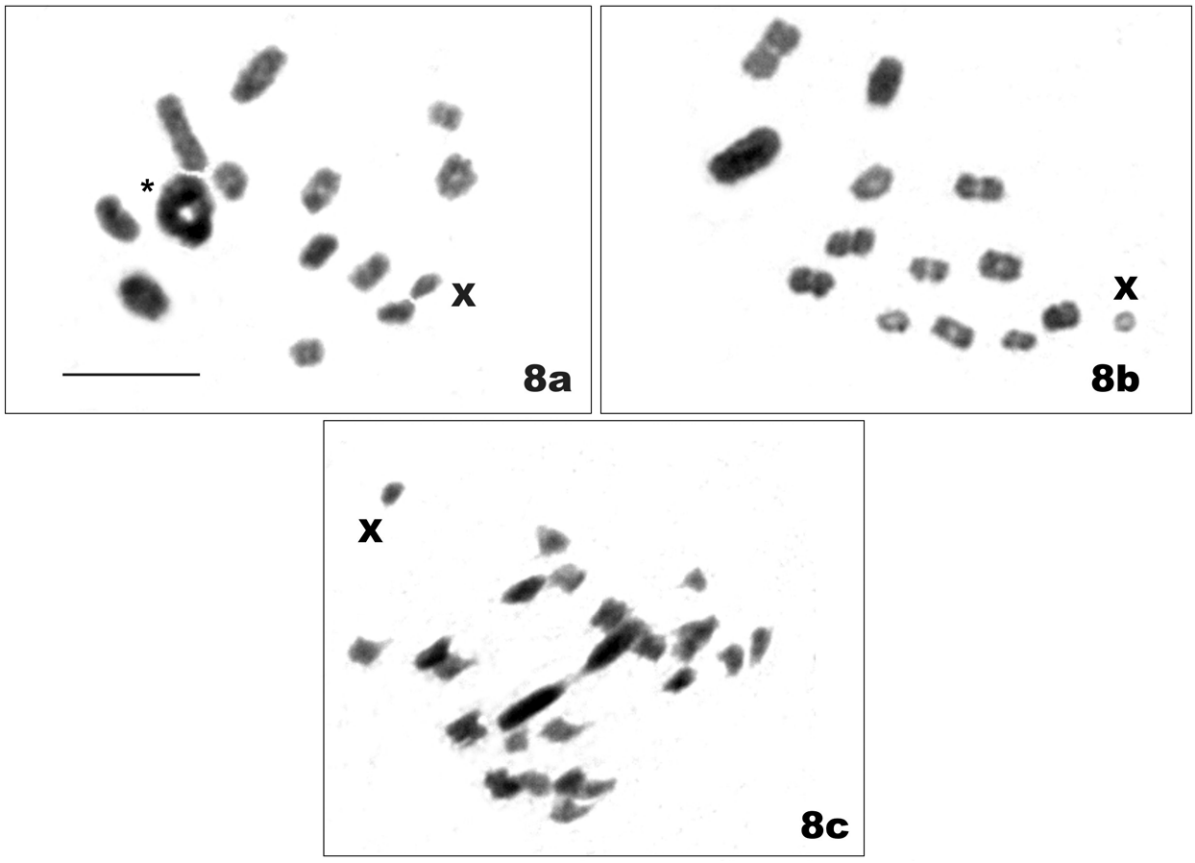

Fig. 8. Meiotic chromosomes of *Mulsantereum abruzicum*: a, b – diakinesis/metaphase I (a) and metaphase I (b), n = 13 + X; c – anaphase I. Bar indicates 10 μm.

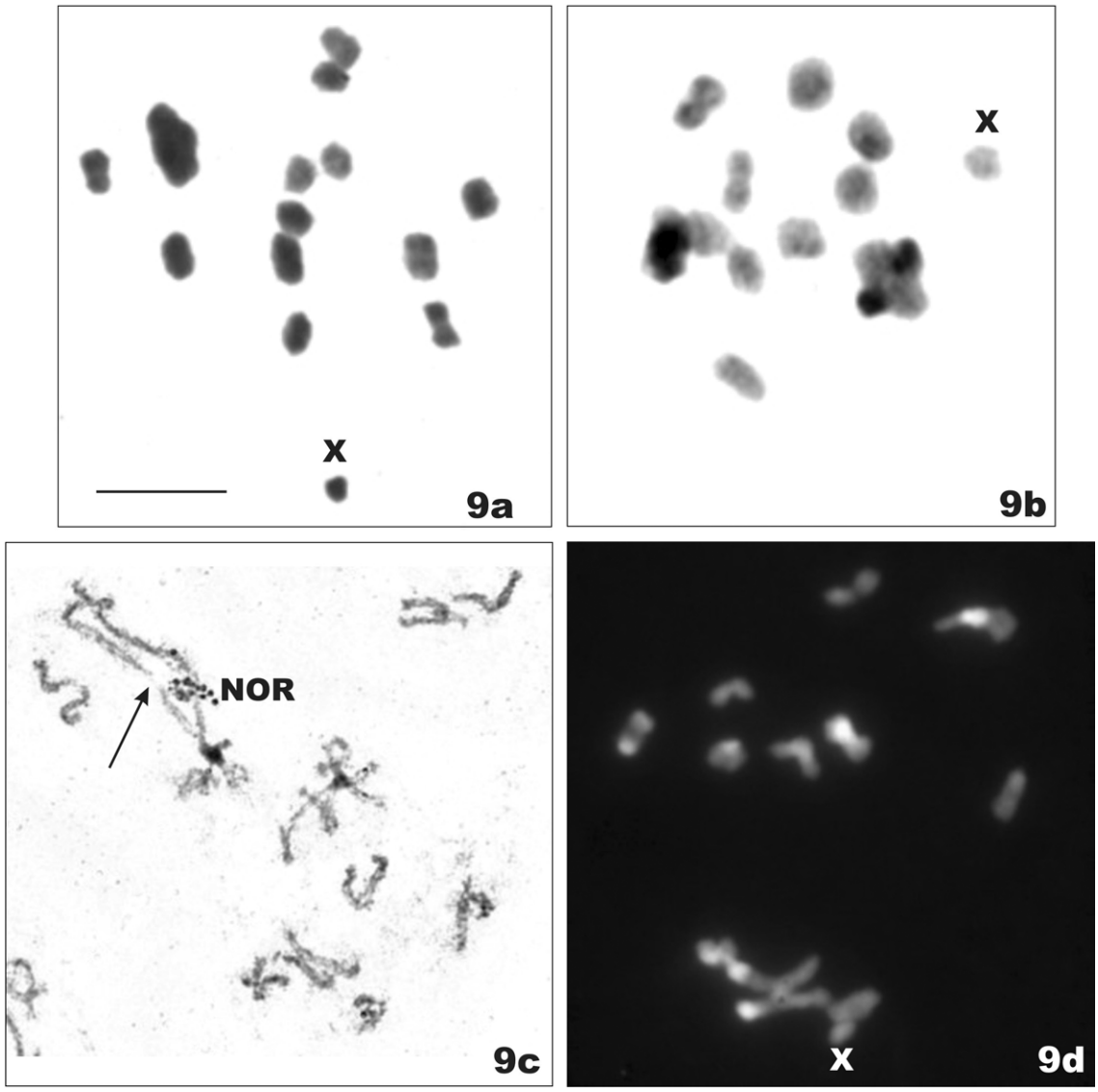

Fig. 9. Meiotic chromosomes of *Mycterodus colossicus*: a – diakinesis/metaphase I, n = 13 + X; b – C-banded diakinesis; c – silver-stained diplotene; d – DAPI-banded diakinesis. Arrow indicates gap on one homologue of the largest bivalent (c). Bar indicates 10 μm.

nucleolar remnants adjacent to one homologue of the largest bivalent, which suggests an active NOR is interstitially located there; the other homologue has a gap in this region (Fig. 9c).

M. (*Mycterodus*) *etruscus* Dlabola, 1980: 2n = 26 + X (Fig. 10).

Testes each consist of 16 follicles. Whilst this species differs from *M. colossicus* in testis structure its karyotype is similar. However, this karyotype is not as well studied as that of *M. colossicus*. Fig. 10 shows a diakinesis/metaphase I transition with 13 autosomal bivalents, one of which is very large, and the double-stranded X chromosome. The sex chromosome tends to be located apart from the bivalents and appears to be highly condensed and similar in size to the small-sized half-bivalents in the nucleus.

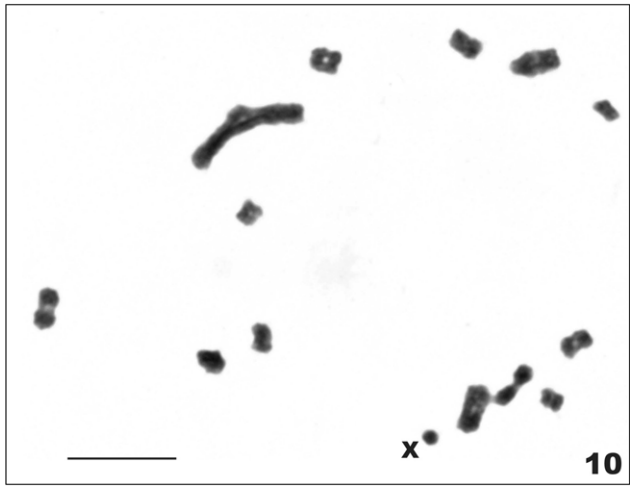

Fig. 10. Meiotic chromosomes of *Mycterodus etruscus*: diakinesis/metaphase I transition, n = 13 + X. Bar indicates 10 μm.

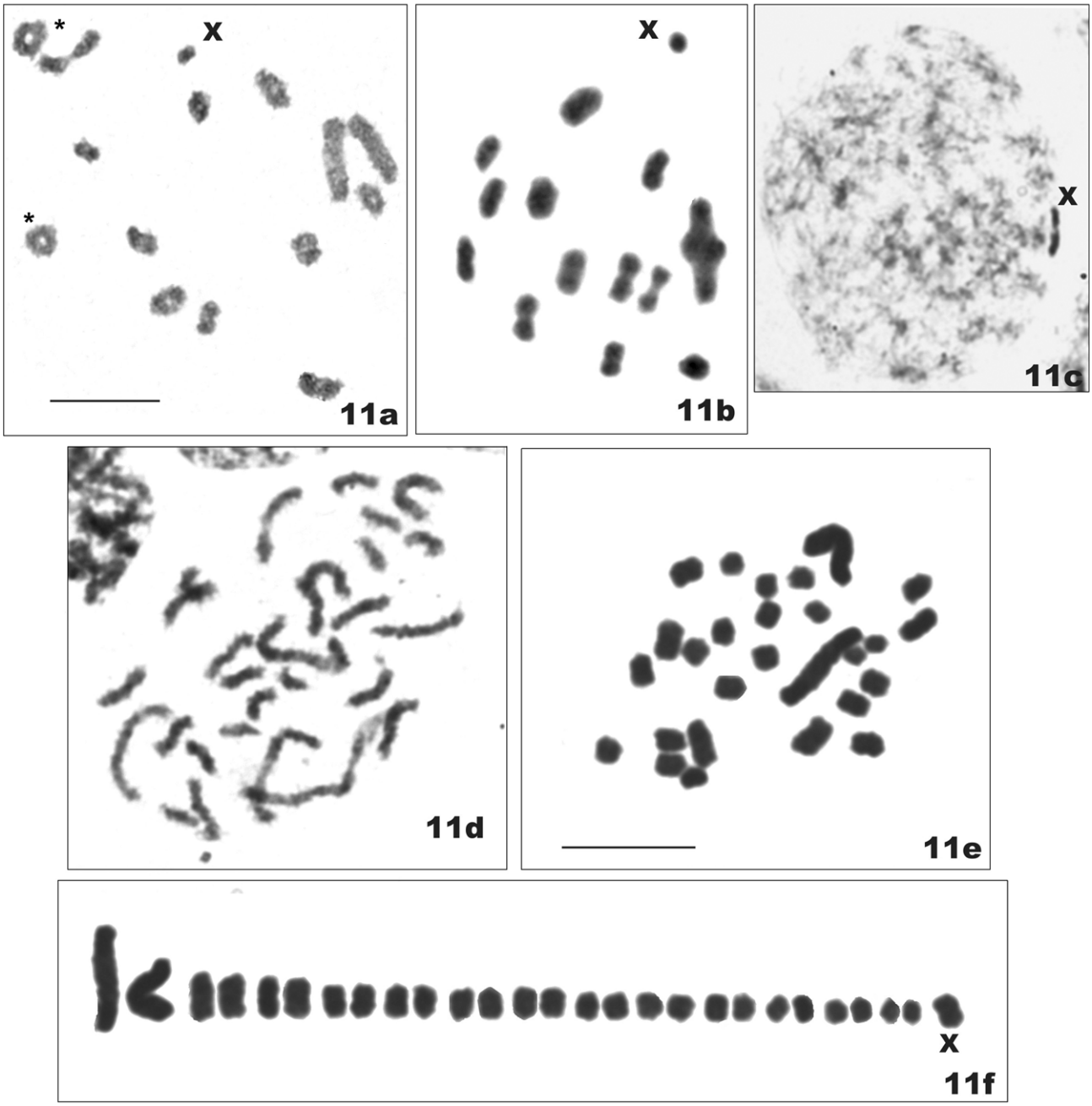

Fig. 11. Meiotic (a–c) and mitotic (d–f) chromosomes of *Mycterodus intricatus*: a, b – diakinesis (a) and metaphase I (b), n = 13 + X; c – premeiotic interphase; d – spermatogonial prometaphase, 2n = 27 (26 + X); e, f – spermatogonial metaphase (e) and karyogram (f) prepared from the same metaphase. Asterisks indicate bivalents with two chiasmata (a). Bar indicates 10 μm.

M. (*Mycterodus) intricatus* Stål, 1861: 2n = 26 + X (Fig. 11a–f).

Testes each consist of 20 follicles. During meiotic stages, 13 bivalents and an X chromosome univalent were observed; one of the bivalents is noticeably larger than the others and the remaining bivalents are of a gradually decreasing size; rather large X is similar in size to the large half-bivalents (Fig. 11a, b). The large size of the heteropycnotic X chromosome is well seen in the premeiotic interphase presented in Fig. 11c. At diakinesis, presented in Fig. 11a, the majority of bivalents have a single chiasma but two small ring bivalents suggest the presence of two chiasmata and the largest bivalent has separate homologous chromosomes. Spermatogonial mitoses in one male allowed a more precise analysis of chromosome sizes than could be obtained from pictures of meiosis. There are 27 chromosomes at prometaphase (Fig. 11d) and metaphase (Fig. 11e, f). All the chromosomes have a well-defined holokinetic structure. It is difficult to see the homologous chromosomes with the exception of the longest pair, whose distinctive size was especially evident at metaphase; it was not possible to distinguish the X chromosome at prometaphase or metaphase.

***Scorlupaster* Emeljanov, 1971**

Published data. Absent.

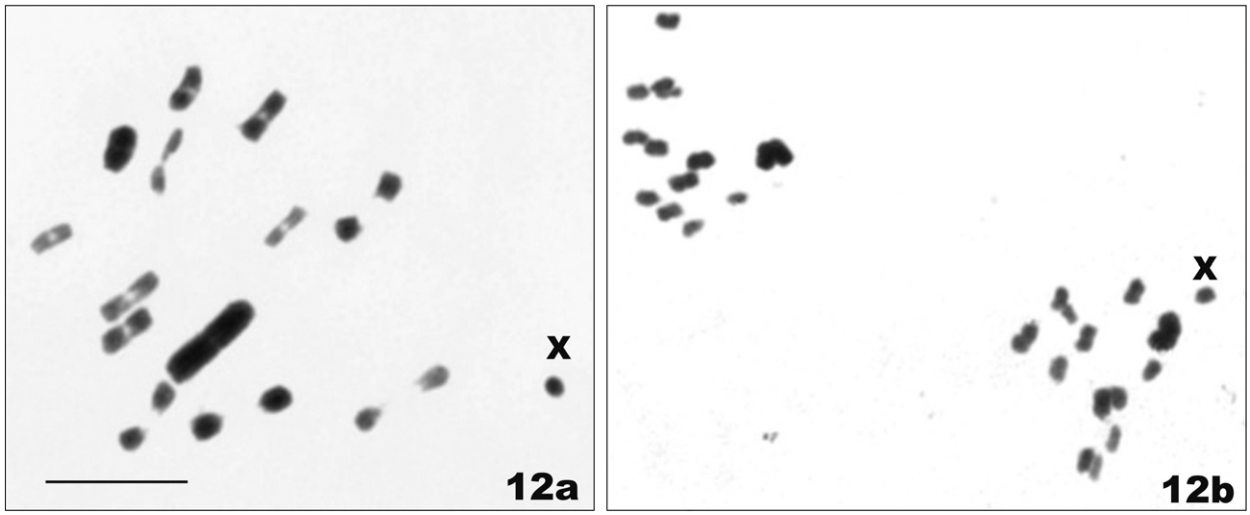

Fig. 12. Meiotic chromosomes of *Scorlupaster asiaticum*: a – metaphase I, n = 13 + X; b – two daughter metaphase II cells, n = 13 and 14 respectively. Bar indicates 10 μm.

S. asiaticum (Lethierry, 1878): 2n = 26 + X (Fig. 12a, b).

Testes each consist of 9 follicles. Fig. 12a shows a spermatocyte metaphase I with 13 autosomal bivalents, one of which is noticeably larger than the others, and a double-stranded X chromosome univalent. The latter appears to be highly condensed and similar in size to the medium-sized half-bivalents in the nucleus studied. At this stage, the X is located far from the bivalents. During anaphase I the sex chromosome undergoes segregation, and on two daughter metaphase II plates there are 13 and 14 chromosomes, respectively, with the X chromosome still located peripherally (Fig. 12b)

***Scorlupella* Emeljanov, 1971**

Published data. Absent.

S. discolor (Germar, 1821): 2n = 26 + X (Fig. 13a–c).

Testes each consist of 6 follicles. During meiotic stages in males, 13 bivalents and an X chromosome univalent were observed suggesting 2n = 26 + X (Fig. 13a, b). One of these bivalents is markedly longer than the others, which gradually decrease in size. The X chromosome is

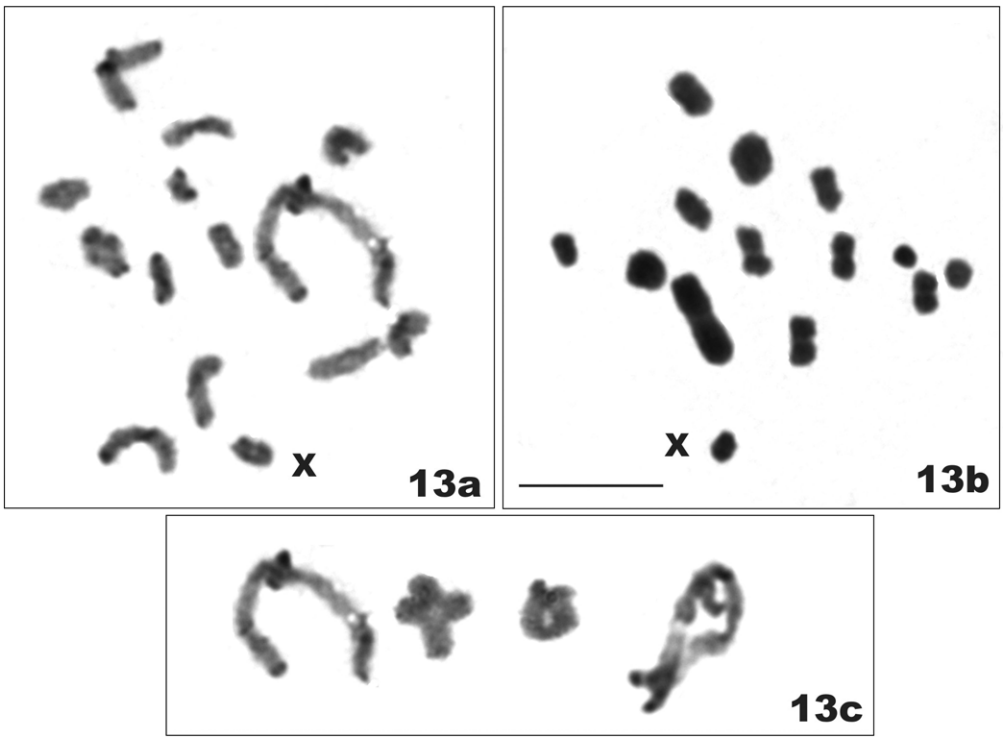

Fig. 13. Meiotic chromosomes of *Scorlupella discolor*: a, b – diakinesis (a) and metaphase I (b), n = 13 + X; c – the largest bivalent with one subterminal chiasma; one interstitial chiasma; two chiasmata; three chiasmata, respectively (from left to right). Bar indicates 10 μm.

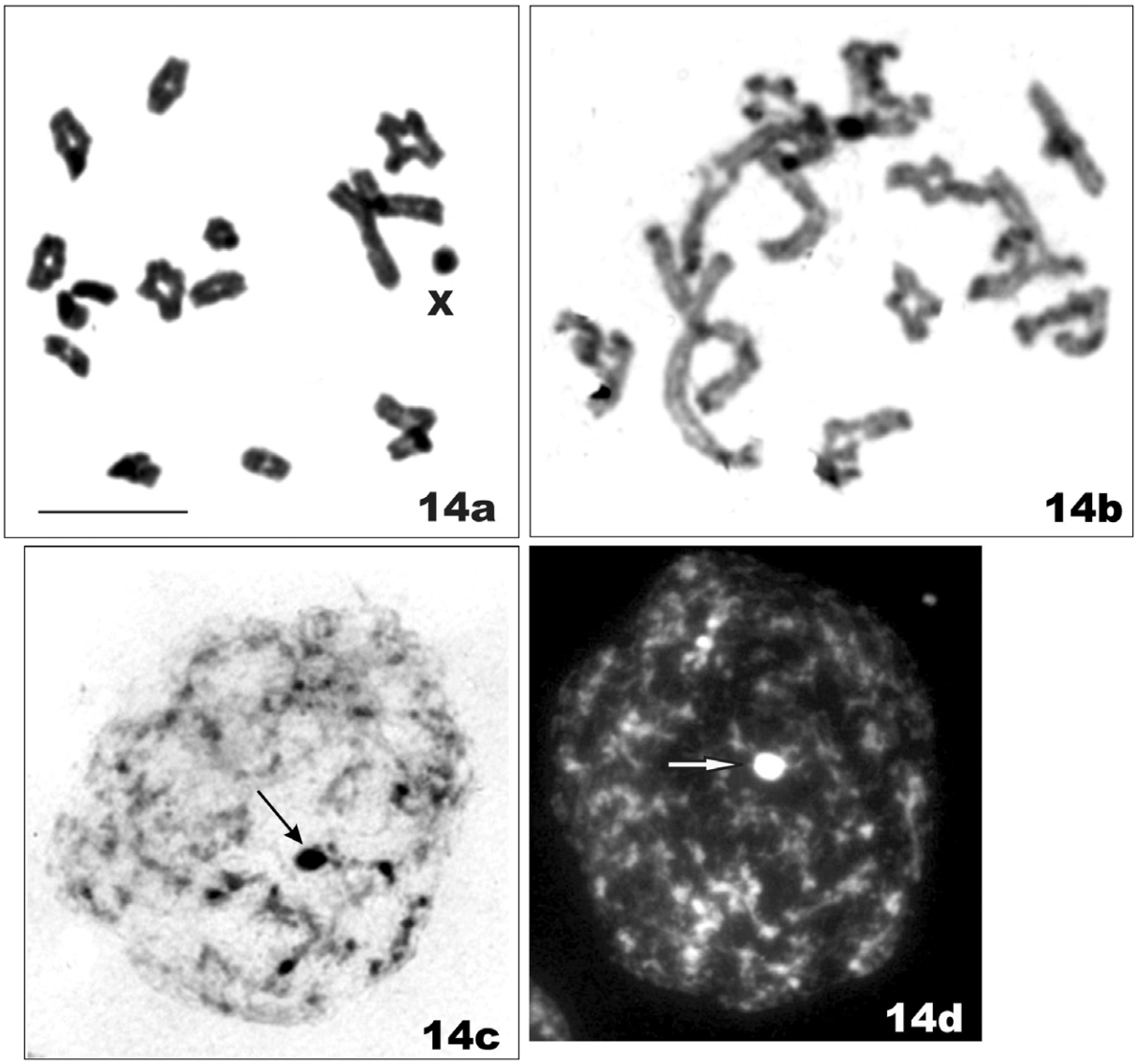

Fig. 14. Meiotic chromosomes of *Zopherisca tendinosa tendinosa*: a – diakinesis, n = 13 + X; b – C-banded diplotene; c, d – C-banded (c) and DAPI-banded (d) premeiotic interphase. Arrows show C-and DAPI-banded X chromosome (c, d). Bar indicates 10 μm.

similar in size to the medium-sized half-bivalents and located among the bivalents at diakinesis (Fig. 13a) but far from them at metaphase I (Fig. 13b). There is a single chiasma on the majority of the bivalents, with up to three chiasmata on the largest bivalent at diakinesis (Fig. 13c).

***Zopherisca* Emeljanov, 2001**

Published data. *Z. penelopae* (Dlabola, 1974): 2n = 26 + X; 24. fol./per testis (Maryańska-Nadachowska et al., 2006) and *Z. tendinosa skaloula* (Spinola, 1839): 2n = 26 + X; 30 fol./per testis (Maryańska-Nadachowska et al., 2006: determined as *Z. tendinosa*).

Z. tendinosa tendinosa (Spinola, 1839): 2n = 26 + X (Fig. 14a–d).

Testes each consist of 28 follicles. At diakinesis there are 13 autosomal bivalents, one of which was noticeably larger than the others, and an X chromosome univalent is round in form and located among the bivalents at this stage (Fig. 14a) in the division of spermatocyte cells. The bivalents mainly have a single subterminal or interstitial chiasma. Conventional C-banding of cells at diplotene revealed small C-heterochromatic blocks on the majority of the bivalents (Fig. 14b). The C-banding of premeiotic interphase cells revealed small scattered C-blocks and a large heterochromatic body, probably the X chromosome (Fig. 14c). After DAPI-banding the sex chromosome in interphase nuclei fluoresced brightly (Fig. 14d).

DISCUSSION

Chromosome complements

The karyotypes and male meiosis of 14 species of the planthopper tribe Issini (Fulgoroidea: Issidae) were studied using conventional staining, occasionally C- and fluorochrome DAPI-banding, and silver nitrate impregnation. In addition, the testis structure was determined for males of every species in terms of the number of seminal

follicles, except for *F. limbatus*. The species investigated belong to the genera *Bubastia* (2 species), *Falcidius* (2), *Kervillea* (2), *Mulsantereum* (1), *Mycterodus* (3), *Scorlupaster* (1), *Scorlupella* (1), *Zopherisca* (1) from the subtribe Hysteropterina, and *Latissus* (1) from the subtribe Issina. The male karyotype is remarkably similar in all these species with 13 having 2n = 26 + X and one, *Falcidius limbatus*, having 2n = 24 + neo-XY. Noteworthy is the neo-XY system reported here for the first time in the family Issidae. Including the previously published data on the tribe Issini (reviewed by Maryańska-Nadachowska et al., 2006) a total of 36 species and subspecies from 20 genera have now been cytogenetically examined. The majority of these species are from the subtribe Hysteropterina, with six species belonging to the subtribes Issina (3) and Agalmatiina (3). The males of all the species studied have a karyotype of 2n = 26 + X, except *Brahmaloca* sp. and *Latilica maculipes* with 2n = 24 + X and *Falcidius limbatus* with 2n = 24 + neo-XY. There is good evidence that the chromosome number of the tribe Issini is much less variable than one would expect, at least theoretically, because the chromosomes are holokinetic. Chromosome rearrangements did not play an important role in species diversification in this group.

The basic male karyotype of 2n = 26 + X is considered to be phylogenetically ancestral in the family Issidae, at least in the tribe Issini, where it occurs in all the three studied subtribes – Issina, Hysteropterina, and Agalmatiina (Maryańska-Nadachowska et al., 2006; present paper). The male karyotype of 2n = 24 + X found in *Brahmaloca* sp. and *L. maculipes* (Issini) (Parida & Dalua, 1981; Maryańska-Nadachowska et al., 2006) could have been derived by fusion of two autosomal pairs of the basic karyotype. These species are not closely related and their chromosome complements might have arisen independently during evolution, and consequently it is likely that different pairs of autosomes were involved in the fusion (Maryańska-Nadachowska et al., 2006).

The XX/X0 is the most common sex chromosome system in Auchenorrhyncha; however, some species have the XX/XY system. In the great majority of cases the XX/XY is a neo-system, which is best exemplified by the tribe Almanini of the fulgoroid family Dictyopharidae (Kuznetsova, 1985; Kuznetsova et al., 2009a). The neo-XY system originated from the more simple ancestral X0 type (White, 1973). In the case of *Falcidius limbatus,* studied here, the origin of its chromosome complement of 2n = 24 + XY could be due to a fusion between an autosome and an ancestral X of an original X0 system in an ancestral male chromosome complement of 2n = 26 + X. Then, the homologue of this autosome would become a neo-Y chromosome. Clearly, the derived karyotype should have one pair autosomes less than the ancestral one. Because of its secondary karyotype, *F. limbatus* appears to be an advanced taxon within the genus *Falcidius*. This suggestion is confirmed by morphological data. According to the revision of the genus *Falcidius* (Gnezdilov & Wilson, 2008), *F. limbatus* belongs to the *F. apterus* species group, which is the most advanced. The heteromorphy and the occurrence of recombination in the neo-XY pair in *F. limbatus* indicate that it is probably a relatively recent structure. It may be mentioned here that the ancestral autosomal pair, which was involved in the origin of the *F. limbatus* neo-XY, was probably one of the larger pairs but not the largest (see next paragraph).

Chromosome markers

The identification of chromosome markers in groups with holokinetic chromosomes is of crucial importance because of the lack of primary constrictions (the centromeres) on holokinetic chromosomes (Grozeva & Nokkala, 2003; Mandrioli et al., 2003; Angus et al., 2004; Golub et al., 2004; Kuznetsova et al., 2009b; Nguyen et al., 2010). Consequently, the chromosomes within a karyotype are extremely difficult to identify reliably, and rearrangements of holokinetic chromosomes can only be detected if different banding techniques are used. These techniques occasionally provide information, which is not detected by classical cytogenetics and provide useful tools for the study of karyotype structure and evolution in holokinetic groups. This is most commonly done using C-banding for revealing the content and distribution of constitutive heterochromatin (C-blocks); fluorescent banding by the AT-specific compound DAPI and the antibiotic CMA_3, which binds preferentially with GC-rich chromatin commonly encountered at rDNA sites; silver staining; and, in recent years, fluorescent in situ hybridization (FISH) used for the most part with rDNA probes, which localize the sites of ribosomal RNA genes. Only in some mainly economically important organisms, the investigation of holokinetic chromosomes is currently carried out using modern approaches such as immunofluorescence techniques and different modifications of FISH, including chromosome painting, BAC-FISH technique, and GISH/FISH mapping of genes (Mandrioli et al., 2003; Mandrioli & Borsatti, 2007; Marec et al., 2010), however these techniques are not yet developed or available for Auchenorrhyncha.

Over the past 20 years, a number of studies have used conventional banding techniques, such as C-, AgNOR-, DAPI-, CMA_3-banding, to study auchenorrhynchan karyotypes (Noda & Tatewaki, 1990; Perepelov et al., 2002; Kuznetsova et al., 2003, 2009b; Maryańska-Nadachowska et al., 2008). Although these studies achieved their intended objectives (i.e., they revealed chromosome markers), their limited taxonomic representation failed to provide a comprehensive insight into the comparative cytogenetics of the group.

Within the tribe Issini the modal chromosome complement of 2n = 26 + X looks uniform in terms of morphology, displaying no detectable interspecific differences in chromosome size and structure. This complement is characterized by a very large autosomal pair, with the remaining pairs decreasing in size; however, three or four pairs are clearly larger than the others at mitosis. The X chromosome is similar in size to the larger bivalents (it is more difficult to estimate the physical size of the X chromosome at meiosis than at mitosis).

In a previous paper (Kuznetsova et al., 2009b), the first evidence was provided that even if species of the tribe Issini share the same chromosome number and a similar general plan of structure, they differ considerably in banding patterns. The chromosome banding techniques used in both studies (Kuznetsova et al., 2009b; present study) revealed a number of discriminatory landmarks on the chromosomes that provide insights into similarities and differences inherent in the 2n = 26 + X karyotype of different species.

There is broad agreement that holokinetic chromosomes have little C-heterochromatin, and few C-bands, which when present, tend to be located at the telomeric regions (see for example Collet & Westerman, 1984; Camacho et al., 1985; Blackman, 1987; Papeschi, 1988; Grozeva & Nokkala, 2003; Lanzone & Souza, 2006). However, neither structural nor behavioural features of holokinetic chromosomes set limits on the accumulation of heterochromatin (Kuznetsova et al., 1998). In support of this assertion there is a great deal of heterochromatin in some holokinetic species (Papeschi, 1991; Kuznetsova et al., 1997; Angus et al., 2004; Bressa et al., 2005) and some specialized chromosomes like sex chromosomes and B chromosomes, which are sometimes completely or almost completely heterochromatic (Blackman, 1990; Papeschi, 1995; Maryańska-Nadachowska, 2004; Nechaeva et al., 2004; Angus et al., 2004; Grozeva et al., 2004).

Little is known about the molecular composition of heterochromatin in holokinetic chromosomes. A few studies indicate that the molecular composition of the heterochromatin in holokinetic chromosomes might be similar to that in monocentric chromosomes, i.e., consisting of satellite DNAs (Spence et al., 1998; Mandrioli et al., 2003). On the other hand, most Lepidoptera have a heterochromatic W chromosome composed of either retrotransposons (Abe et al., 2005) or various interspersed W-accumulated repeats (Traut et al., 2007; Marec et al., 2010). It is thought that holokinetic chromosomes differ only slightly or not at all, from monocentric chromosomes in the amount of constitutive heterochromatin but differ in the distribution C-heterochromatin (Kuznetsova et al., 2009b). It is well known that monocentric chromosomes have pericentromeric and telomeric C-bands and very rarely interstitial C-bands. Holokinetic chromosomes lack pericentromeric heterochromatin since they do not have a localized centromere, however, they have both telomeric and interstitial C-blocks. As may be inferred from the studies on the Issini (Kuznetsova et al., 2009b; present paper), C-blocks are difficult to visualize in highly condensed chromosomes at meiotic metaphases but are visible in prophase chromosomes. Several bivalents are easily identified in meiotic cells of all the species and have very characteristic banding patterns. One of these bivalents is the largest in which there are telomeric and interstitial blocks, with the latter probably coincident with sites of NORs. One other marker bivalent is a middle-sized bivalent with conspicuous interstitial C-blocks. It is concluded that the above mentioned characters could be inherent in the ancestral chromosome complement of the tribe Issini.

C-banding is known to be a useful tool for identifying differences in holokinetic karyotypes of related species (Rebagliati et al., 2003; Golub et al., 2004; Grozeva et al., 2004; Lanzone & Souza, 2006), including those of the Auchenorrhyncha (Perepelov et al., 2002; Kuznetsova et al., 2003; Maryańska-Nadachowska et al., 2006). The species of the tribe Issini also appear to differ in the amount and distribution of C-heterochromatin. For example, both *Hysteropterum albaceticum* and *Agalmatium bilobum* have a large amount of C-heterochromatin, but there is clearly more in the *H. albaceticum* karyotype (Kuznetsova et al., 2009b). Also in the current study, *Bubastia obsoleta* was found to have more C-heterochromatin than *Zopherisca tendinosa tendinosa*. From the above, it might be assumed that the gain and loss of heterochromatin are mechanisms of karyotype evolution in the tribe Issini.

There is also an interspecific difference in the X chromosome, which lack detectable C-blocks in some species (*A. bilobum*, *Latissus dilatatus*, *Mycterodus collosicus*), but in others (*H. albaceticum*, *Bubastia obsoleta*, *B. saskia*, *Falcidius doriae*) there is a conspicuous interstitial C-block (Kuznetsova et al., 2009b; present study). In terms of taxonomy it is necessary to draw inferences from these differences. However, C-banding suggests that taxonomically related species with the same chromosome number do not have identical karyotypes due to the accumulation of many rearrangements since diverging from a common ancestor.

In the Issini species studied herein, the presence of C-heterochromatin was revealed by DAPI-banding, however CMA_3-banding was not used. In *Hysteropterum albaceticum*, C-regions are equally enriched with AT and GC base pairs, except for the NORs (rDNA sites), which are GC-rich/CMA_3-positive (Kuznetsova et al., 2009b). It is also noteworthy that the NORs are similarly GC-rich in other auchenorrhynchan species (Kuznetsova et al., 2003; Maryańska-Nadachowska et al., 2008) suggesting that this pattern is characteristic of the Auchenorrhyncha as a whole.

In the Issini species, NORs are invariably present at an interstitial position on the largest pair of autosomes (Kuznetsova et al., 2009b; present study: *L. dilitatus* and *M. collosicus*). It is noteworthy that this uniformity accords with the above suggestion about the ancestry of the chromosome complement of 2n = 26 + X in the Issini. In addition, verification comes from the undoubtedly derived karyotype of *F. limbatus* (2n = 24 + neo-XY), in which the largest pair of autosomes also seems to have NORs, as indicated by the gaps, with the NOR sites located interstitially on this pair.

Chiasmata

The species studied have one or occasionally two crossover points per bivalent with the only exception being the three chiasmata observed in a single cell of *Scorlupella discolor*. Previously, a cell with a three chiasmatic large bivalent was reported in *H. albaceticum*; while all other

issid species studied have one-two chiasmata per bivalent (Maryańska-Nadachowska et al., 2006; Kuznetsova et al., 2009b; present paper). Halkka (1964) was the first to suggest that the low number of chiasmata (estimated to be one-two from cytological analysis) is the standard pattern for holokinetic bivalents. Even in the very large bivalent of the psyllid species *Psylla* (= *Baeopelma*) *foersteri* (Flor, 1861) (Homoptera: Sternorrhyncha: Psylloidea), which may have resulted from the fusion of five or six "ordinary" chromosomes, at most only two chiasmata occur (Suomalainen & Halkka, 1963). Recently, Nokkala et al. (2004) report that three chiasmata can occasionally form on the very large bivalent of *Baeopelma foersteri*. However, they conclude that more than two chiasmata on a holokinetic bivalent obstructs the regular course of meiosis and results in the elimination of cells of this type, which results in a strong selection against the formation of more than two chiasmata on holokinetic bivalents. Although bivalents with multiple chiasmata are occasionally observed in holokinetic groups, including the Auchenorrhyncha (Tian & Yuan, 1997; Kuznetsova et al., 2003, 2009a, b; Maryańska-Nadachowska et al., 2008; present paper), they are only observed in cells at stages up to metaphase I of spermatogenesis and therefore, the fate of these cells remains unknown.

Testis structure

The phylogenetic importance for Auchenorrhyncha of the number of testicular follicles has been repeatedly discussed (Emeljanov & Kuznetsova, 1983; Kirillova, 1989; Bednarczyk, 1993; Kuznetsova, 1985; Kuznetsova et al., 1998, 2009a; D'Urso et al., 2005). For example, this character is shown to be constant at the level of tribes and/or subfamily within the fulgoroid families Delphacidae and Dictyopharidae, with the changes in this character correlated with their evolution (Kirillova, 1989; Kuznetsova, 1985; Kuznetsova et al., 2009a). In contrast, in the Issini the structure of testes is highly variable suggesting rapid evolution of follicle number, so this character is not a good basis for considering phylogenetic relationships across the tribe (Maryańska-Nadachowska et al., 2006). The follicles vary in number from 4 in *Palmallorcus punctulatus* (Rambur, 1840) to 30 in *Zopherisca tendinosa skaloula* Gnezdilov & Drosopoulos, 2006 (Maryańska-Nadachowska et al., 2006: determined as *Z. tendinosa*), and the numbers reported here (6–28) fall within the same range. It is important to look at the variation in number, which occurs below the generic level, between species. In most genera follicle number is constant (10 per testis in the genera *Bubastia*, *Hysteropterum* and *Kervillea*; 11 in *Agalmatium*; 13 in *Issus*), while in several genera it varies insignificantly between species (16, 18, and 20 in *Mycterodus*; 24, 28 and 30 in *Zopherisca*) (Maryańska-Nadachowska et al., 2008; present paper). Thus, within genera this character varies little, if at all, suggesting there is normally strong stabilizing selection. Differences in follicle number per testis found in three hitherto studied representatives of the genus *Zopherisca*, i.e. 28 in *Z. tendinosa tendinosa* (present paper), 24 in *Z. penelopae*, and 30 in *Z. tendinosa skaloula* (Maryańska-Nadachowska et al., 2006) allow some taxonomic inferences to be made. Since the number of follicles per testis appears to be a species-specific trait in the tribe Issini, it is suggested that *Z. tendinosa skaloula* be upgrade to species level: *Z. skaloula* Gnezdilov & Drosopoulos, 2006, stat. n.

One can assume that a series of follicle numbers, varying by steps of one or two within genera, evolved from some particular ancestral number. In a previous paper (Maryańska-Nadachowska et al., 2006) it was speculated that 10 is the most typical number for the Issidae, at least the tribe Issini, and this current study provides support for this view, since half the species studied have 10 follicles per testis. It is possible that this value is the plesiomorphic state for the tribe Issini and a greater or more rarely a smaller number of follicles an apomorphic character. On the basis of what has been said above, it is suggested that the variation in the number of the testicular follicles might be phylogenetically significant in the Issini. However, a greater number of taxonomic groups need to be sampled in order to evaluate whether this pattern can be used as a phylogenetic character in this group.

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