

Variability in the karyotypes, testes and ovaries of planthoppers of the families Issidae, Caliscelidae, and Acanaloniidae (Hemiptera: Fulgoroidea)

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Abstract. The aim of this study was to obtain information on the karyotypes, testes and ovaries of three fulgoroid families, mainly in the Issidae but also in the Caliscelidae and Acanaloniidae. For the Issidae, the data is for 19 species belonging to 11 genera of the subtribes Issina (2 species, 1 genus), Hysteropterina (14 species, 9 genera) and Agalmatiina (3 species, 2 genera) of the tribe Issini. The male karyotypes are shown to be quite uniform across the tribe, with $2n = 26 + X$ in all species studied except *Latilica maculipes* (Melichar, 1906) with $2n = 24 + X$. The modal karyotype, $2n = 26 + X$, matches the most probable ancestral state in the Fulgoroidea. In the majority of cases the number of seminal follicles in males and ovarioles in females are stable within but fairly variable among the species, the modal value of the follicle number is 10 per testis. Contrary to what might be expected from other fulgoroid families, such as the Dictyopharidae and Delphacidae, the variability in these characters revealed neither regular trends nor evident correspondence with the taxonomy of Issidae. In the Caliscelidae, all species studied had testes consisting of 6 follicles each and karyotypes of $2n = 24 + X$ and $26 + XY$, respectively, in 3 and 1 species. The only representative studied of the Acanaloniidae, *Acanalonia bivittata* (Say, 1825), had $2n = 24 + X$ and 13 follicles in its testis. The variability in all the characters investigated is discussed and compared to other fulgoroid families, primarily to the most extensively studied families, Delphacidae and Dictyopharidae.

INTRODUCTION

The Auchenorrhyncha possess holokinetic chromosomes, which are generally accepted to facilitate karyotype evolution through fragmentation or fusion of chromosomes. However, the chromosome numbers (of 181 species from 114 genera and 15 families; Kuznetsova et al., 1998) in the superfamily Fulgoroidea remain within reasonably narrow limits from 19 (X0) to 37 (X0) in males, each of these numbers occurring only in one and two species, respectively, although the overwhelming majority of the species have $2n = 26$ (XY), 27 (X0) and 29 (X0), with the latter predominating (Halkka, 1959; Kirillova, 1986; Emeljanov & Kirillova, 1991; Kuznetsova et al., 1998). However, these values are greatly skewed towards the families Dictyopharidae and Delphacidae, with over 120 karyotyped species (Kuznetsova, 1985; Kirillova, 1991). In the Dictyopharidae (42 species studied), changes in karyotype as well as in number of seminal follicles in males are of taxonomic and phylogenetic importance (Kuznetsova, 1985). In the Delphacidae karyotypes of 79 species are recorded. For all other families (e.g. Cixiidae – 12 species; Derbidae – 12 species; Achilidae – 20 species; Flatidae – 16 species) the data are fragmentary or absent (Achilixiidae and Kinnaridae).

The family Issidae Spinola, 1839 is distributed worldwide and is one of the most diverse families of the Fulgoroidea. The systematics of the family was recently

revised, but is not yet fully established (Emeljanov, 1990, 1999; Gnezdilov, 2002, 2003a). At present, some taxa, previously referred to the Issidae (e.g. the subfamilies Caliscelinae Amyot & Serville, 1843 and Acanaloniinae Amyot & Serville, 1843), are treated as distinct families (Emeljanov, 1999; Yang & Chang, 2000; Gnezdilov, 2003a, b). According to the recent revision of Gnezdilov (2003a), the Issidae sensu stricto contains one subfamily with five tribes (Issini Spinola, 1839, Parahiraciini Cheng, & Yang 1991, Hemisphaerini Melichar, 1906, Thioniini Melichar, 1906 and Colpopterini Gnezdilov, 2003). The tribe Issini is split into 3 subtribes (Gnezdilov, 2002, 2003b): Issina Spinola, 1839, Hysteropterina Melichar, 1906 and Agalmatiina Gnezdilov, 2002.

In the Issidae sensu Gnezdilov, only the karyotypes of three species, *Brahmaloca* sp., *Colpoptera* sp. and *Sivaloca damnosa* Chou & Lu (Parida & Dalua, 1981; Halkka, 1961) and the ovaries and/or testes of two species, *Agalmatium bilobum* (Fieber) and *Issus coleoptratus* (Fabricius), have been studied (Dufour, 1833; Emeljanov & Kuznetsova, 1983).

In this paper the karyotypes, number of testicular follicles and ovarioles of 19 species of Issidae are reported. Some new data on the families Caliscelidae and Acanaloniidae are also included. The results are discussed and compared with the available data on other fulgoroid families, primarily that on the most extensively studied families Delphacidae and Dictyopharidae.

MATERIAL AND METHODS

The localities and number of specimens that were karyologically and anatomically studied are given in Tables 1 and 2. New data concern 19 species of Issidae from 12 genera of the subtribes Issina (2 species, 1 genus), Hysteropterina (14 species, 9 genera) and Agalmatiina (3 species, 2 genera) of the tribe Issini; 3 species of Calliscelidae from 2 genera; and 1 species of Acanaloniidae. Nomenclature and taxonomy follow Gnezdilov (2003a, b, 2005).

Freshly caught adult specimens were fixed in a 3 : 1 ethanol-glacial acetic acid solution. Both karyotypes and testis structure were studied in males and only the number and form of ovarioles in females. Slides for cytogenetic studies were prepared by the squash method. Testes were extracted from the abdomen in a drop of 45% acetic acid, testicular follicles were separated, counted and squashed under cover glasses. Cover glasses were

removed by a dry-ice technique, slides were air-dried and analyzed under phase contrast at 400×. The best slides were stained using a standard Feulgen-Giemsa procedure (Grozeva & Nokkala, 1996). They were 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 slides were additionally stained in 4% Giemsa in Sørensen's buffer pH 6.8 for 20 min. The slides were rinsed briefly with distilled water, air-dried and mounted in Entellan. The preparations were examined under a Leica MM 4000 microscope at 1000× and photomicrographs taken using a Nikon DS-U1 camera.

The diakinesis and first metaphase stages of meiosis and rarely the metaphase stage of mitosis of about 1000 nuclei from a total of 59 specimens (50 males and 9 females) were examined. The number of specimens studied ranged from 1 to 6, but mostly 1 to 3 per species (Tables 1 and 2).

TABLE 1. List of the Issidae (Issinae) species, male diploid chromosome numbers, numbers of follicles per testis and ovarioles per ovary, collection localities, number and sex of specimens. The species for which there is no data are indicated with “—”.

Taxa	2n	Number of		Collection locality, number and sex of specimens	References
		male follicles	female ovarioles		
Issini Spinola					
Issina Spinola					
<i>Issus coleoptratus</i> (Fabricius, 1781)	26 + X	13	—	Spain, 2♂	present data
	—	20	—	France	Dufour, 1833
<i>I. lauri</i> Ahrens, 1814	26 + X	13	13*	Italy (Sicily), 2♂, 3♀	present data
Hysteropterina Melichar					
<i>Hysteropterum albaceticum</i> Dlabola, 1983	26 + X	10	—	Spain, 3♂	present data
<i>H. vasconicum</i> Gnezdilov, 2003	26 + X	10	—	Spain, 3♂	present data
<i>H. dolichotum</i> Gnezdilov & Mazzoni, 2004	26 + X	—	—	Spain, 2♂	present data
<i>Tshurtshurnella pythia</i> Dlabola, 1979	26 + X	12	7	Greece, 3♂, 2♀	present data
<i>Mycterodus (Semirodus) pallens</i> (Stål, 1861)	26 + X	18	9	Greece, 1♂, 1♀	present data
<i>Zopherisca penelopae</i> (Dlabola, 1974)	26 + X	24	—	Greece, 3♂	present data
<i>Z. tendinosa</i> (Spinola, 1839)	26 + X	30	—	Greece, 1♂	present data
<i>Latilica maculipes</i> (Melichar, 1906)	24 + X	10	—	Italy, 2♂	present data
<i>Bubastica taurica</i> (Kusnezov, 1926)	26 + X	10	—	Russia, 1♂	present data
<i>Bergevinium ?malagense</i> (Matsumura, 1910)	26 + X	9	—	Spain, 2♂	present data
<i>Palaeolithium distinguendum</i> (Kirschbaum, 1868)	26 + X	8–11**	8	Spain, 5♂, 1♀	present data
<i>Palmallorcus balearicus</i> (Dlabola, 1982)	26 + X	9–11***	—	Spain, 3♂	present data
<i>P. nevadense</i> (Linnavuori, 1957)	26 + X	10	—	Spain, 2♂	present data
<i>P. punctulatus</i> (Rambur, 1840)	26 + X	4?	—	Spain, 1♂	present data
<i>Sivaloka damnosa</i> Chou & Lu, 1985	26 + X	—	—	China	Tian et al., 2004
Agalmatiina Gnezdilov					
<i>Agalmatium bilobum</i> (Fieber, 1877)	—	11	8	Russia, Greece,	Emelyanov & Kuznetsova, 1983; present data
	26 + X	11	8	Italy, 3♂, 2♀	
<i>A. flavescens</i> (Olivier, 1791) group	26 + X	11	—	Spain, 2♂	present data
<i>Tingissus tangirus</i> (Matsumura, 1910)	26 + X	10	6	Spain, 4♂	present data
Thioniini Melichar					
<i>Brahmaloca</i> sp.	24 + X	—	—	India	Parida & Dalua, 1981
Colpopterini Gnezdilov					
<i>Colpoptera</i> sp.	26 + X	—	—	Panama	Halkka, 1961

* One of the females had respectively 13 and 12 follicles in different ovaries; ** Number of follicles varied between males and between testes of a male as follows: 7/13, 8/8, 8/11, 9/9, 9/11; *** Number of follicles varied between males and between testes of a male as follows: 9/10, 10/10, 11/11.

TABLE 2. List of species of Caliscelidae and Acanaloniidae, male diploid chromosome numbers, numbers of follicles per testis and ovarioles per ovary, collection localities, number and sex of specimens. The species for which there is no data are indicated with “—”.

Taxa	2n	Number of		Collection locality and number of specimens	References
		male follicles	female ovarioles		
Caliscelidae Amyot et Serville					
<i>Ommatidiotus dissimilis</i> (Fallén, 1806)	24 + X	—	—	Finland	Halkka, 1957, 1959; Ivanov, 1928
	—	6	10		
<i>Ommatidiotus</i> sp.	24 + X	—	—	Greece, 1 ♂	present data
<i>Caliscelis bonellii</i> (Latreille, 1807)	26 + XY	6	—	Spain, 2 ♂	present data
<i>C. nero</i> Fennah, 1967	24 + X	6	—	South Africa, 1 ♂	present data
Acanaloniidae Amyot et Serville					
<i>Acanalonia bivittata</i> (Say, 1825)	24 + X	—	—	USA	Boring, 1907: <i>Poeciloptera</i> , <i>Amphiscepa</i> ; present data
	24 + X	13	—	USA, 1 ♂	

RESULTS

Karyotypes

The diploid chromosome number the tribe Issini showed little variation (Table 1). Males of all but one species had $2n = 27$ ($26 + X$) (Figs 1–19). Also, only a few mitoses were recorded in adults in all species (Fig. 16b). The course of meiosis in 19 species conformed to the general auchenorrhynchan pattern described by Halkka (1959). Diakineses and first metaphases (MI) each revealed 13 autosomal bivalents and a univalent X chromosome (Figs 1–8a, 9, 11–16a, 17–19a). At the second metaphases (MII) both 13 or 14 ($13 + X$) chromosomes appeared (Figs 8b, 19b). A primary constriction was not observed on the chromosomes, therefore they are holokinetetic, as is the case in Hemiptera as a whole. The karyotypes of the species are structurally broadly similar to one another. The first bivalent stands out because of its fairly large size, whereas all other bivalents form a series of decreasing size. The X chromosome is one of the smaller elements of the karyotype. The X tends to be positioned at a distance from the bivalents both in MI and MII (Figs 4, 11, 19a, b), which seems to be characteristic of the Auchenorrhyncha as a whole (Halkka, 1959). The bivalents each show a single chiasma: terminal/subterminal and, very rarely, interstitial (Figs 7, 9, 17), but not the first bivalent, which had two chiasmata in 0.6% of the cells analysed (Fig. 15a). It was difficult to distinguish NORs and nucleoli in MI, but very often the largest bivalent displayed gaps that were probably NORs (Figs 2, 7, 9, 15a, 17). Males of *Latilica maculipes* (Melichar) have a different karyotype: $2n = 25$ ($24 + X$). In this species MI revealed 12 bivalents and a univalent X chromosome (Fig. 10). Just as in other issid species, the first bivalent is the largest element of the karyotype, all other bivalents form a series decreasing in size and the X chromosome is one of the smaller elements of the karyotype.

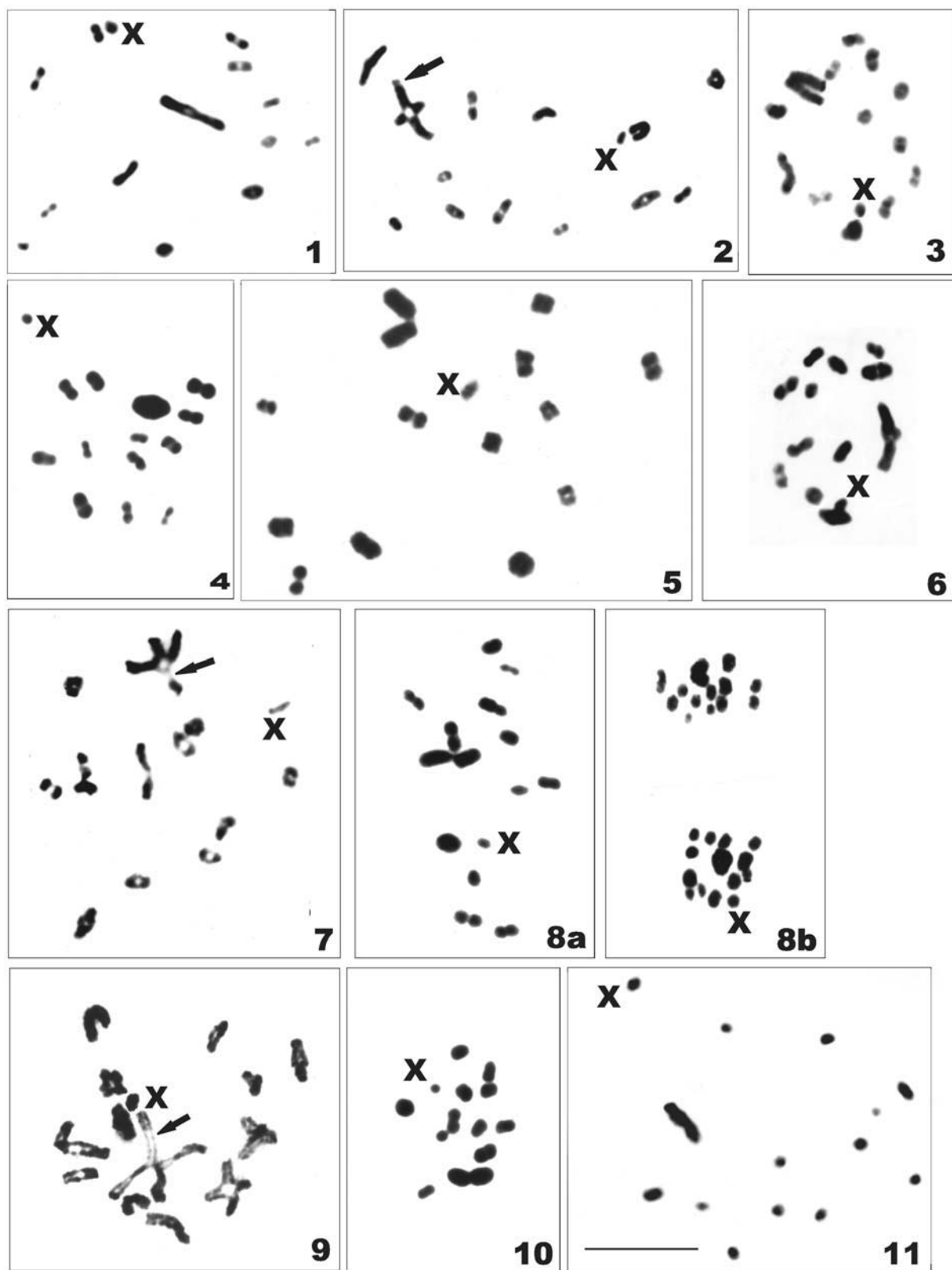
Males of *Ommatidiotus* sp. and *Caliscelis nero* Fennah from the family Caliscelidae and *Acanalonia bivittata* (Say) from the family Acanaloniidae had $2n = 25$ ($24 + X$) (Table 2). No mitoses were found in adults. In each species, MI revealed 12 bivalents and a univalent X chromosome (Figs 20, 22, 23a, b). The first bivalent is the

largest element, all other bivalents form a series decreasing in size and the X chromosome is one of the smaller elements of the karyotype. One of the bivalents is very large while the others form a series decreasing in size, one of these being positively heteropycnotic. The bivalents are chiasmatic with one subterminal or interstitial chiasma each, except for the largest bivalent that shows two chiasmata in separate nuclei (Fig. 22). The largest bivalent and sometimes one of the middle-sized bivalents have gaps, most probably NORs (Fig. 23a). In the meiotic cells of *Caliscelis bonellii* (Latreille) of the two males examined, there were 14 bivalents, including one heteromorphic bivalent (Figs 21a, b). In the latter, the character of the connection between chromosomes is obscure. This bivalent may be thought of as consisting of X and Y chromosomes and the male karyotype of this species to be $2n = 28$ ($26 + XY$).

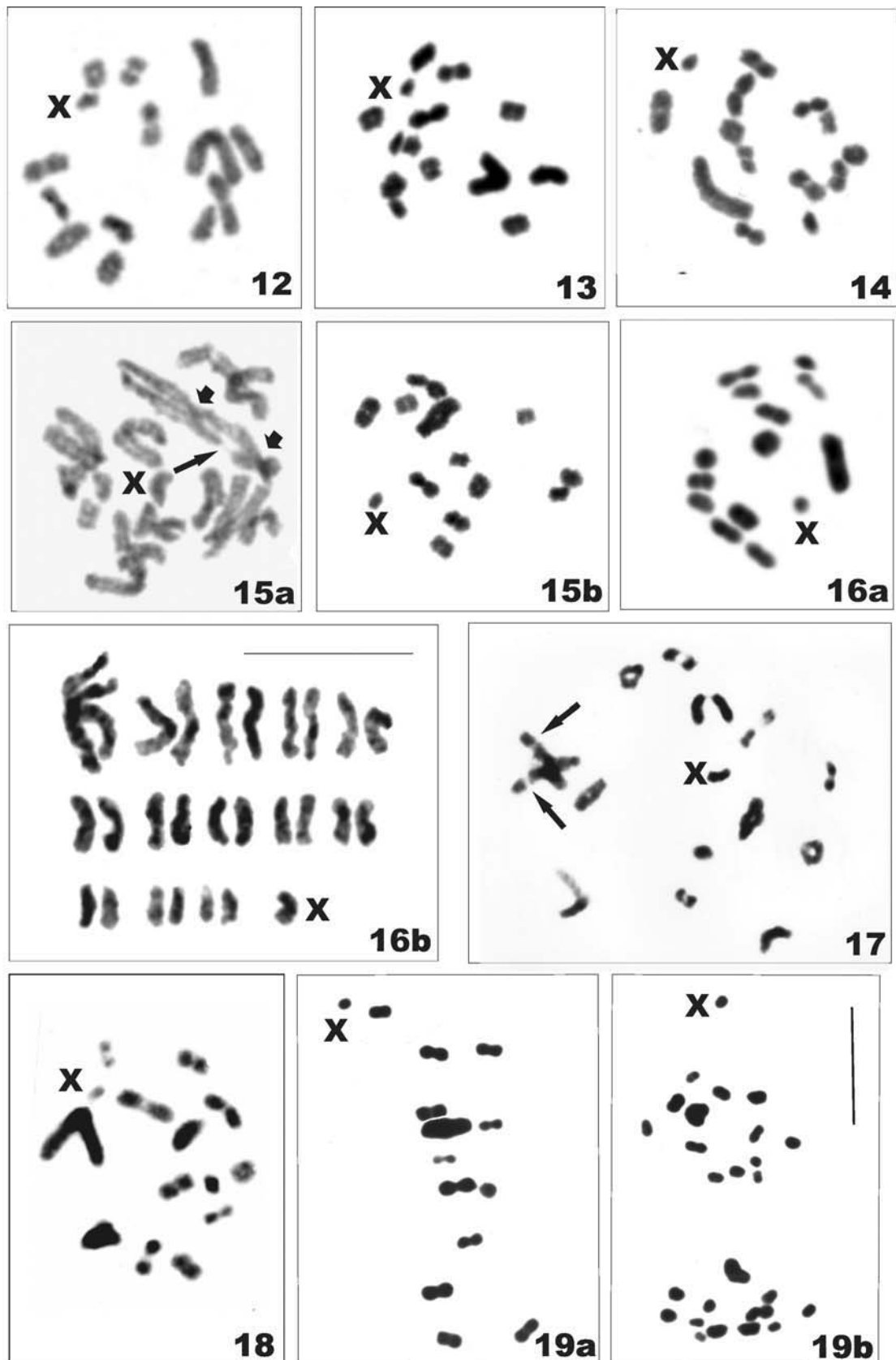
Testes

The internal reproductive system in males consists of paired testes, each with long fusiform follicles (testis tubes) covered by a yellow sheath, a cylindrical seminal duct with a more or less well developed widened part serving as a seminal vesicle and an extended accessory gland. The number of seminal follicles was determined for males of all but three species (Tables 1 and 2).

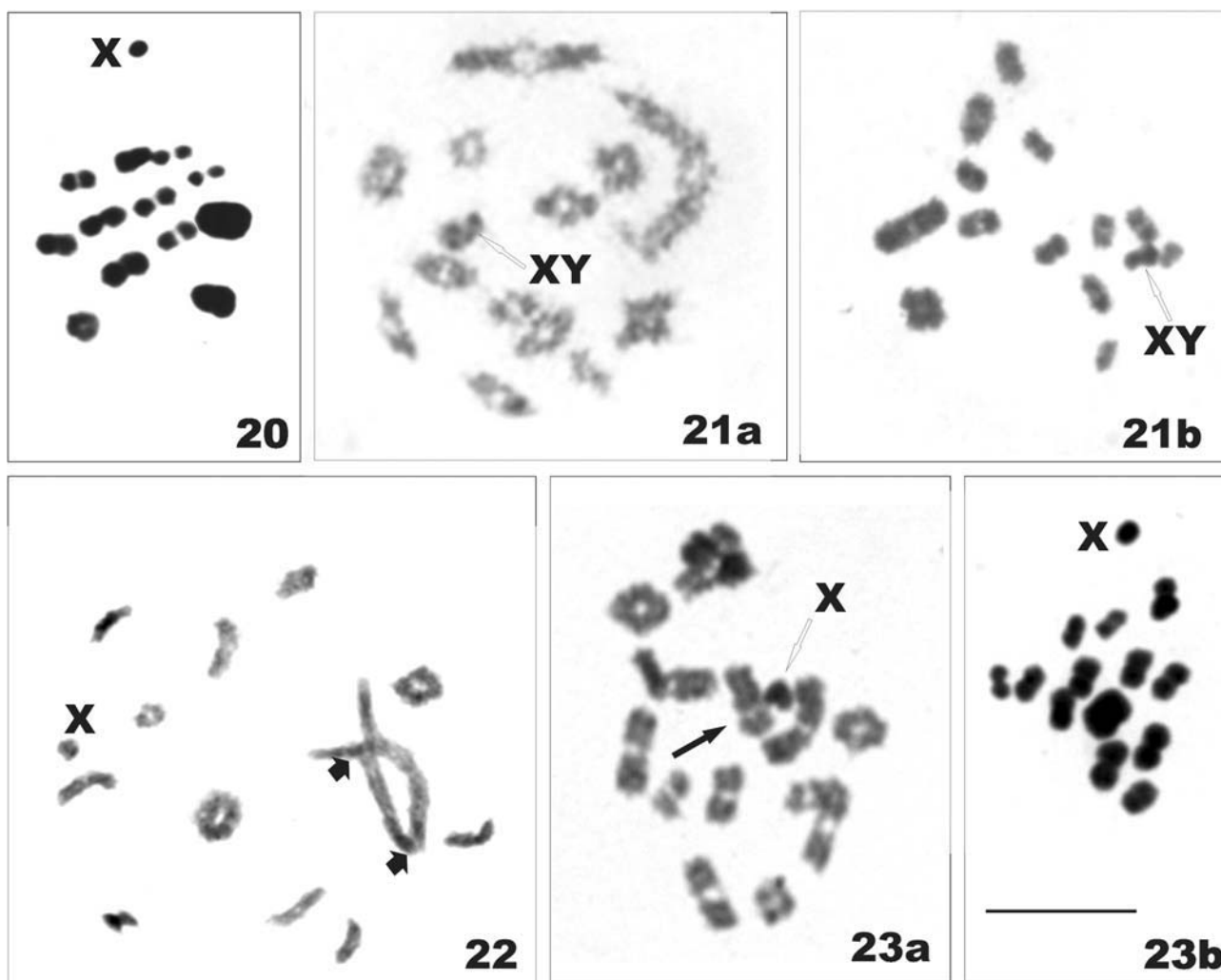
In the Issini there is great variation in follicle number between species and sometimes between males of one species and even between the testes of a male (Table 1). Follicle numbers (in a testis) range from 4 (*Palmallorcus punctulatus* (Rambur) to 30 (*Zopherisca tendinosa* (Spinola)). The modal value of 10 is found in 8 species of 6 genera. Other values include 9 (1 species), 11 (2 species), 12 (1 species), 13 (2 species), 18 (1 species) and 24 (1 species). Within the genera *Issus* Fabricius, *Hysteropterum* Amyot et Seville, *Agalmatium* Emeljanov and *Tingissus* Gnezdilov, closely related species have the same number of follicles, i.e. 13, 10, 11 and 10, respectively, per testis. In the genus *Zopherisca* Emeljanov males of two species have 24 and 30 follicles, respectively. Differences in follicle number were also evident between species of the genus *Palmallorcus* Gnezdilov. In *P. punctulatus*, the only male studied had 4 follicles in



Figs 1–11. Meiotic and mitotic chromosomes of males of Issidae. 1 – *Issus coleoptratus*, MI, $n = 13 + X$; 2 – *I. lauri*, MI, $n = 13 + X$; 3 – *Hysteropterum albaceticum*, MI, $n = 13 + X$; 4 – *H. vasconicum*, MI, $n = 13 + X$; 5 – *H. dolichotum*, MI, $n = 13 + X$; 6 – *Tshurtshurnella pythia*, MI, $n = 13 + X$; 7 – *Mycterodus pallens*, MI, $n = 13 + X$; 8a, b – *Zopherisca penelopae*, a – MI, $n = 13 + X$; b – two daughter MIIs, $n = 13$ and $n = 13 + X$; 9 – *Zopherisca tendinosa*, diakinesis, $n = 13 + X$; 10 – *Latilica maculipes*, MI, $n = 12 + X$; 11 – *Bubastica taurica*, MI, $n = 13 + X$. The arrows indicate gaps. Bar equals 10 μm .



Figs 12–19. Meiotic and mitotic chromosomes of males of Issidae. 12. *Bergevinium ?malagense*, MI, $n = 13 + X$; 13 – *Palaeolithium distinguendum*, MI, $n = 13 + X$; 14 – *Palmallorcus balearicus*, MI, $n = 13 + X$; 15a, b – *P. nevadense*, a – diakinesis; b – MI, $n = 13 + X$; 16a, b – *P. punctulatus*, a – MI, $n = 13 + X$; b – karyogram prepared from a mitotic metaphase, $2n = 26 + X$; 17 – *Agalmatium bilobum*, MI, $n = 13 + X$; 18 – *Agalmatium flavescens* group, MI, $n = 13 + X$; 19a, b – *Tingissus tangirus*, a – MI, $n = 13 + X$; b – two daughter MIIs, $n = 13$ and $n = 13 + X$. The short arrows indicate chiasmata; the long arrows indicate gaps. Bar equals 10 μm .



Figs 20–23. Meiotic and mitotic chromosomes of males of Caliscelidae (20–22) and Acanaloniidae (23). 20 – *Ommatidiotus* sp., MI, $n = 12 + X$; 21a, b – *Caliscelis bonellii*, a – diakinesis, b – MI, $n = 13 + XY$; 22 – *C. nero*, MI, $n = 12 + X$; 23a, b – *Acanalonia bivittata*, two MIs, $n = 12 + X$. The short arrows indicate chiasmata; the long arrows indicate gaps. Bar equals 10 μm .

each testis, but in two males of *P. nevadense* (Linnavuori) there were 10 follicles per testis. In *P. balearicus* (Dlabola), one male had 10 follicles in each testis and another 11 follicles. A different specimen contained 9 and 10 follicles in each testes. In *Palaeolithium distinguendum* (Kirschbaum), each of five males examined had a specific testis structure as follows: 8/8, 8/10, 8/11, 9/9, 9/11.

There are always 6 follicles per testis in males of *Caliscelis bonellii* (1 male examined) and *C. nero* (2) of the family Caliscelidae and *Acanalonia bivittata* (1) of the family Acanaloniidae (Table 2).

Ovaries

Of the female reproductive system only the ovaries were examined. The data obtained are for 6 species (6 genera) of the family Issidae (Table 1). As with seminal follicles, there was intraspecific variation in the number of ovarioles in females in this group. The number of ovarioles varied from 6 (*Tingissus tangirus* (Matsumura)) to 13 (*Issus lauri* Ahrens) per ovary with all values between 6 and 9. In one of three females examined of *I.*

lauri, one ovary contained 13 ovarioles and another only 12.

DISCUSSION

The issid species examined in this study all belong to the tribe Issini. The diploid chromosome numbers and sex-chromosome systems are found to be highly uniform across the tribe, with $2n = 27$ ($26 + X$) in males, with the exception of *Latilica maculipes* (of the subtribe Hysteropterina), which has $2n = 25$ ($24 + X$). The same karyotype $2n = 24 + X$ is reported in male *Brahmaloca* sp. from the tribe Thioniini (Parida & Dalua, 1981). Two further species, *Colpoptera* sp. and *Sivaloka damnosa*, respectively referred to the tribes Colpopterini and Issini, have $2n = 26 + X$ (Halkka, 1961; Tian et al., 2004). Thus, altogether the data is for the tribes Issini (20 species, 13 genera, 3 subtribes), Thioniini (1 species) and Colpopterini (1 species) (Table 1). Based on the frequency of the $2n = 26 + X$ karyotype among the species studied to date, this chromosome number is the most typical for the Issidae. This karyotype occurs in separate representatives of the fami-

lies Nogodinidae, Tropiduchidae, Flatidae and Ricaniidae (Kirillova, 1986; Kuznetsova et al., 1998) but has not been found in the Caliscelidae or Acanaloniidae, however, very few species of these groups have been studied (Table 2). It is worth noting that the modal karyotype $2n = 26 + X$ in the Issidae is probable the ancestral karyotype of the Fulgoroidea, based primarily on evidence from the most extensively studied families, Delphacidae (in particular the most primitive subfamily Asiracinae) and Dictyopharidae (the most primitive tribe Ranissini of the subfamily Orgeriinae) (Kuznetsova et al., 1998; Tian et al., 2004).

So far, efforts to resolve family-level relationships based on chromosome data have only been successful in the Dictyopharidae (Kuznetsova, 1985). In this family the karyotypes are known for many species and species groups, and almost each tribe is characterized by a peculiar chromosome number, which is particularly true of the subfamily Orgeriinae. In the subfamily Dictyopharinae, $2n = 28 + X$ was found in the tribes Orthopagini and, with rare exceptions (Tian et al., 2004), in the tribe Dictyopharini, but $2n = 36 + X$ was found only in the tribe Scoloptini (Halkka, 1959; unpubl. data). The comparatively more advanced subfamily Orgeriinae is particularly well studied, with all representatives of the tribe Orgeriini having $2n = 26 + X$ and those of the tribe Almanini $2n = 24 + \text{neo XY}$ (Kuznetsova, 1985). It is noteworthy that this contrasts with that in all the other fulgoroid families, including the most comprehensively studied family Delphacidae, where the great majority of species have $2n = 28 + X$, with all the derived chromosome numbers showing no correlation with taxonomy (Kirillova, 1991). The same seems to be true for the Issidae for which the presently available chromosome data provide no taxonomic clues since only the derived karyotype, $2n = 24 + X$, is known, occurring in two unrelated genera. However, the presently available data is only for the tribe Issini, which shows uniformity of karyotypes. As shown above, the tribes within the Dictyopharidae differ from each other in karyotype, thus, further investigations of the Issidae are needed in order to gain a better insight into the karyotypic differentiation of the family.

In the Caliscelidae, three of the four species studied have $2n = 24 + X$ (Table 2). In the genus *Caliscelis* de Laporte two species have different karyotypes, $2n = 24 + X$ in *C. nero* and $2n = 26 + XY$ in *C. bonellii*. The secondary sex chromosome system XY in *C. bonellii* indicates its karyotype is derived. The character of the connection between X and Y chromosomes at MI, whether they are held together by a chiasma or not, and the origin of the Y chromosome in this species are obscure. The question of the origin of a Y chromosome in insect groups with a X0 sex chromosome system as the ancestral system is discussed by Nokkala et al. (2003) with reference to the Psylloidea (Hemiptera). They suggested that the Y chromosome could have evolved from a mitotically stable B chromosome, which first integrated into an achiasmatic segregation system with the X chromosome and later became fixed in the karyotype as a Y chromosome. In the

Fulgoroidea, *Phantia subquadrata* (Herrich-Schäffer) and *Ph. christofii* de Rusiecka from the family Flatidae also display X0 and XY systems, respectively (unpubl. data; Kuznetsova & Kirillova, 1990). Since both species have the same number of autosomes (26), the XY system in *Ph. christofii* could not have originated through X-autosome fusion, therefore the XY system in this species is undoubtedly not a neo XY. In regard to *C. bonellii*, studies on different populations of the species and tracing the behaviour of the X and Y chromosomes during meiosis are needed to verify the status and origin of the Y chromosome.

The initial chromosome number, direction and mechanisms of karyotype evolution in the Issidae cannot be resolved at present. While the modal karyotype, $2n = 26 + X$ may seem to be plesiomorphic (see above), this is not necessarily the case, since commonality does not need to be translated into ancestrality. Similarly, it can be assumed that the plesiomorphic karyotype is $2n = 24 + X$, as is undicated by its occurrence both in the Issidae, including the most primitive tribe Thioniini and Caliscelidae. However, to avoid a false interpretation, the polarization of the karyotype changes in Issidae must be inferred using a phylogenetic approach.

In contrast to the very stable karyotype, the structure of testes in the Issidae is highly variable, the variability does not exhibit either regular trends or evident correspondence with taxonomy. This is also the case in some other fulgoroid families, including the families Nogodinidae and Flatidae, for which only fragmentary data are available (Kuznetsova et al., 1998). In the Issidae, closely related species may sometimes have the same follicle number (*Hysteropterum*: 10) and sometimes differ from each other in testis structure (*Zopherisca*, *Palmallorcus*). Follicle number varies from 4 in *Palmallorcus punctulatus* to 30 in *Zopherisca tendinosa*, the genus *Zopherisca* being unique in having the highest numbers for the family, 30 and 24, in the two species studied. In the Issidae, the most frequent number is 10, occurring in 6 species belonging to 5 unrelated genera. In males of *Palaeolithium distinguendum* and *Palmallorcus punctatus*, the number of follicles varies between testes, but the total number in a male is of the order of 20, i.e. 10 per testis. *Issus coleoptratus* as well as *I. lauri*, each with two males examined, show 13 follicles in each testis, however, Dufour reported 20 follicles in each testis for male *I. coleoptratus* as early as 1833. The differences are too significant to suggest a counting error, so Dufour (1833) was most likely dealing with a different species.

The Dictyopharidae and the Delphacidae are the most extensively studied fulgoroid families in terms of testis structure. In all the evolutionarily advanced subfamilies of Delphacidae, males invariably have 3 follicles per testis, whereas the primitive subfamily Asiracinae has 6 follicles per testis (Kirillova, 1989). Testes consisting of 6 follicles are also characteristic of all of the most primitive representatives of the Dictyopharidae, whereas in the more advanced tribe Orgeriini, males invariably display 4 follicles per testis (Kuznetsova, 1985; unpubl. data).

Testes with 6 follicles are considered a plesiomorphy in the Fulgoroidea (Emeljanov & Kuznetsova, 1983). If this is true, then all the Caliscelidae studied have retained the initial testis structure, whereas polymerization of the follicle number has occurred in the Issidae, while *Palmallorcus punctulatus*, with 4 follicles per testis, is the only exception. From the predominance of the value 10 it may be deduced that the Issidae show an initial stage of stabilization of this character with a higher modal value compared to other fulgoroid families, primarily the Delphacidae and Dictyopharidae. In some species inconsistent counts are observed. In the Fulgoroidea as a whole, intraspecific variation is observed only when the number of follicles is high, over 6 per testis (Emeljanov & Kuznetsova, 1983). The same regularity seems to be characteristic of the Psylloidea (Hemiptera), in which follicle number varies from 1 to 5, being very stable at higher taxonomic levels, with variability observed only in species displaying 4 or 5 follicles per testis (Glowacka et al., 1995).

Data on ovary structure are very scarce for both the Issidae (Table 1) and other fulgoroid families (Emeljanov & Kuznetsova, 1983; Kaplin, 1985; Table 2). In the Dictyopharidae, the number of ovarioles in females as well as testicular follicles in males is fairly stable in all species for which this number is known (Emeljanov & Kuznetsova, 1983; unpubl. data). Conversely, in the Issidae, the number of ovarioles is variable and mostly lower than the number of follicles and similarly exhibit no regular trends. The differences in the number of ovarioles and seminal follicles in species as well as a high variability of these characters probably testify that within-family evolution in Issidae is still in progress. However, the available data on the structure of testes and ovarioles and the data on karyotypes are not yet sufficient for making any comprehensive inference about the evolution and systematics of the family Issidae.

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