ORIGINAL ARTICLE

Detection of telomeric sequences and ribosomal RNA genes in holokinetic chromosomes of five jumping plant-lice species: First data on the superfamily Psylloidea (Hemiptera: Sternorrhyncha)

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Abstract. Fluorescence in situ hybridization (FISH) is a technique used to determine the chromosomal position of DNA and RNA probes. The present study contributes to knowledge on jumping plant-lice genomes by using FISH with 18S rDNA and telomeric (TTAGG)$_n$ probes on meiotic chromosomes of *Psylla alni* (2n = 24 + X), *Cacopsylla mali* (2n = 22 + neo-XY and 20 + neo-X$_1$X$_2$Y), *C. sorbi* (2n = 20 + neo-XY), *Baeopelma foersteri* (2n = 14 + X), and *Rhinocola aceris* (2n = 10 + X). This is the first study that has used FISH on the hemipteran superfamily Psylloidea. We found that the chromosomes of all studied species contain the insect-type telomere motif, (TTAGG)$_n$. In *C. mali* and *C. sorbi*, the neo-sex chromosomes originating from autosome-sex chromosome fusions showed no interstitially located clusters of TTAGG repeats, suggesting their loss or inactivation. Similarly, no interstitial (TTAGG)$_n$ clusters were detected in an extremely large autosome pair of *B. foersteri* that most likely originated from a fusion of at least five ancestral chromosome pairs. Clusters of 18S rDNA were detected on the fused and second largest autosome pairs of *B. foersteri* and on one of the large autosome pairs of the remaining species. In *C. mali* and *B. foersteri*, the rDNA clusters were shown to coincide with the NORs as detected by the AgNOR method. Finally, we speculate, based on the obtained FISH markers, on the mechanisms of karyotype evolution of psylloid species differing in chromosome numbers and sex chromosome systems.

INTRODUCTION

The Psylloidea, or jumping plant-lice (Sternorrhyncha), are small sap-sucking hemipteran insects distributed worldwide. They feed on the phloem of vascular plants, with most species developing exclusively on dicotyledonous plants. Most psyllid species are oligophages restricted to one or a few closely related host plants, particularly at the larval stage. Several species are known to be harmful to their host plants; they can act as vectors for various plant diseases, as economically important pests in agriculture and forestry, and as potential biocontrol agents for some invasive plants. Approximately 4000 psyllid species have been described from across every biogeographic region of the world, most of which from tropical and subtropical regions (Burckhardt, 1987; Burckhardt & Ouvrard, 2012; Burckhardt et al., 2014; Percy et al., 2018). The most recent classification of the Psylloidea (Burckhardt & Ouvrard, 2012) recognizes 8 families: Aphalaridae, Carsidaridae, Calophyidae, Homotomidae, Liviidae, Phacopteronidae, Psyllidae, and Triozidae. Until now, approximately 175 psylloid species belonging to 55 genera

of all families, except for the small family Phacopteronidae, have been analyzed cytogenetically (Maryańska-Nadachowska & Głowacka, 2005; Labina et al., 2007; Nokkala et al., 2008, 2013, 2017; for other references see Maryańska-Nadachowska, 2002). Jumping plant-lice are characterized by fairly uniform karyotypes with regard to both chromosome number, which is predominantly 25/26 (male/female), and sex chromosome system, which is of an X(0)/XX (male/female) type in more than 95% of species. Thus, the modal male karyotype of Psylloidea is 2n = 24 + X, with the latter being regarded as ancestral for this superfamily (Maryańska-Nadachowska, 2002). All other karyotypes are attributable to derived conditions, and the variability seen among chromosome numbers and sex chromosome systems suggests that the deviated karyotypes have evolved independently in different lineages. With the exception of 2n = 26 + XY found in the genera *Pachypsylla* Riley, 1883 (Aphalaridae) and *Pauropsylla* Rübsaamen, 1899 (Triozidae) (Walton, 1960; Maryańska-Nadachowska & Głowacka, 2005), the number of autosomes in these karyotypes is lower than the modal number and ranges from

6 to 20 in diploid complements. Several species display an XY sex chromosome system that is usually considered to be derived from the X(0) system by a fusion of the X chromosome with an autosome, whose homologue then became the Y chromosome (Grozeva & Maryańska-Nadachowska, 1995; Maryańska-Nadachowska, 2002). At least two of the species, *Cacopsylla mali* (Schmidberger, 1836) and *C. sorbi* (Linnaeus, 1767), show sex chromosome polymorphism within populations, with males having either a neo-XY or neo-X_1X_2Y system in the former species and either an X(0) or neo-XY system in the latter (Suomalainen & Halkka, 1963; Grozeva & Maryańska-Nadachowska, 1995; Maryańska-Nadachowska & Grozeva, 2001). The chromosomes of jumping plant-lice are holokinetic (Suomalainen & Halkka, 1963): these chromosomes lack physical landmarks such as primary constrictions (the centromeres) and therefore possess very few differentiating features, which greatly confines comparative cytogenetic approaches to this group. In recent years, different chromosome banding techniques (DNA-binding fluorochromes, C- and AgNOR-bandings) and especially fluorescence in situ hybridization (FISH) have made it possible to reveal chromosome-specific DNA markers for Hemiptera insects (reviewed in Mola & Papeschi, 2006; Papeschi & Bressa, 2006; Kuznetsova et al., 2012, 2015a, b; Kuznetsova & Aguin-Pombo, 2015; Grozeva et al., 2015). In the jumping plant-lice species studied thus far, C-banding has not been able to induce clear or regular patterns of longitudinal differentiation along the chromosomes, while the number and location of the nucleolus organizer regions (NORs) seem to be highly uniform: the NORs are always present on one of the medium-sized pairs of autosomes in a sub-terminal position (reviewed in Maryańska-Nadachowska, 2002; see also: Kuznetsova et al., 2003; Labina et al., 2007). As such, the difficulty in acquiring useful results seems to have limited interest in these approaches. FISH, on the other hand, has never been used in cytogenetic investigations of Psylloidea.

The present paper provides the first cytogenetic knowledge on holokinetic chromosomes of Psylloidea studied with FISH technology. The aims were (1) to ascertain whether jumping plant-lice display the insect-type telomere motif, $(TTAGG)_n$, (2) to analyze the chromosomal distribution of 18S rDNA loci in five species belonging to four genera of two families, the Psyllidae and Aphalaridae, and (3) to speculate on the evolutionary trends and mechanisms of karyotype evolution of jumping plant-lice species with different chromosome numbers and sex chromosome systems, based on FISH markers.

MATERIAL AND METHODS

Taxon sampling and fixation

Adult males of five species were used in this study: *Psylla alni* (Linnaeus, 1758), *Cacopsylla mali* (Schmidberger, 1836), *C. sorbi* (Linnaeus, 1767), *Baeopelma foersteri* Flor, 1861, and *Rhinocola aceris* (Linnaeus, 1758). All specimens were collected in May and June in 2015–2017 in Poland (Table 1) and identified by the senior author (AMN). Males were fixed in the field in a freshly mixed ethanol and glacial acetic acid mixture (3 : 1) and refrigerated in the laboratory at –20°C. The testes were extracted from the abdomen, placed on a slide in a drop of 45% acetic acid and squashed. The coverslips were removed with a razor blade after freezing with dry ice, and the slides then dehydrated in fresh fixative (3 : 1) and air dried.

Fluorescence in situ hybridization (FISH)

Probes for 18S rDNA and $(TTAGG)_n$ were prepared and FISH was performed according to Grozeva et al. (2011) with a few modifications, as described in our recent publications dealing with other hemipteran lineages, Auchenorrhyncha (Maryańska Nadachowska et al., 2013), Heteroptera (Kuznetsova et al., 2012; Angus et al., 2017) and Coleorrhyncha (Kuznetsova et al., 2015b). In brief, the 18S rDNA probe was amplified by PCR and labelled with biotin-11-dUTP (Fermentas, Vilnius, Lithuania) using genomic DNA of the true bug *Pyrrhocoris apterus* (Linneus, 1758): an initial denaturation period of 3 min at 94°C was followed by 33 cycles of 30 s at 94°C, annealing for 30 s at 50°C and extension for 1.5 min at 72°C, with a final extension step of 3 min at 72°C. The telomere probe $(TTAGG)_n$ was amplified by PCR and labeled with rhodamine-5-dUTP (GeneCraft, Köln, Germany): an initial denaturation period of 3 min at 94°C was followed by 30 cycles of 45 s at 94°C, annealing for 30 s at 50°C and extension for 50 s at 72°C, with a final extension step of 3 min at 72°C. For primers, see Grozeva et al. (2011). The chromosome preparations were treated with 100 μg/ml RNase A and 5 mg/ml pepsin solution to remove excess RNA and proteins. Chromosomes were denatured in the hybridization mixture containing labelled 18S rDNA and $(TTAGG)_n$ probes with an addition of salmon sperm blocking reagent and then hybridized for 42 h at 37°C. 18S rDNA probes were detected with NeutrAvidin-Fluorescein conjugate (Invitrogen, Karlsbad, CA, USA). The chromosomes were mounted in an antifade medium (ProLong Gold antifade reagent with DAPI, Invitrogen) and covered with a glass coverslip.

The number of males involved in the study ranged from 4 to 7 and the number of prophase/metaphase plates examined ranged from 20 to 34 in different species (Table 1).

AgNOR-banding

Only two species, *C. mali* and *B. foersteri*, were studied by this method. The chromosome slides were incubated with 50% $AgNO_3$ and 1% gelatin developer at 60°C for 7–8 min, rinsed in distilled water, air-dried, and mounted in Entellan (Merck, Darmstadt, Germany).

Table 1. Material studied.

Species	Collection localities	Host plant	Number of males / nuclei
Psylla alni (Linnaeus, 1758)	Poland, Niepołomice, 50°03´25˝N / 20°21´66˝E	*Alnus incana*	4/24
Cacopsylla mali (Schmidberger, 1836)	Poland, Kraków, 50°08´33˝N / 19°91´67˝E	*Malus* sp.	6/22
Cacopsylla sorbi (Linnaeus,1767)	Poland, Kraków, 50°08´33˝N / 19°91´67˝E	*Sorbus aucuparia*	5/20
Baeopelma foersteri Flor, 1861	Poland, Nowa Biała, 49°45´00˝N / 20°15´00˝E	*Alnus glutinosa*	7/27
Rhinocola aceris (Linnaeus, 1758)	Poland, Kraków, 50°8´33˝N / 19°91´67˝E	*Acer platanoides*	5/34

Table 2. Summary of the cytogenetic data on the five studied jumping plant-lice species (Hemiptera: Psylloidea).

Taxa	$2n\male$	Male karyotype formula	Composition of telomeres	Location of NOR	Location of rDNA	References (origin of insects)
Fam. Psyllidae **Subfam. Psyllinae**						
Psylla alni	25	24 + X		A medium-sized autosome pair (AgNOR-banding)*		Suomalainen & Halkka, 1963 (Finland)
	25	24 + X				Maryańska-Nadachowska et al., 1992a, b (Poland)
	25	24 + X	(TTAGG)$_n$		A large autosome pair*	Present study (Poland)
Cacopsylla mali Neo-XY- cytotype	24	22 + neo-XY		A medium-sized autosome pair (AgNOR-banding)*		Maryańska -Nadachowska et al., 1992a: as *Psylla* (Poland) Grozeva & Maryańska-Nadachowska, 1995 (Poland, Finland, Russia)
	24	22 + neo-XY				Maryańska-Nadachowska & Grozeva, 2001 (Poland, Finland, Russia)
	24	22 + neo-XY			A large autosome pair*	Nokkala et al., 2006 (Finland)
	24	22 + neo-XY	(TTAGG)$_n$			Present study (Poland)
Cacopsylla mali Neo-XXY cytotype	23	20 + neo-X$_1$X$_2$Y		A medium-sized autosome pair (AgNOR-banding)*		Grozeva & Maryańska-Nadachowska, 1995 (Poland) Maryańska-Nadachowska & Grozeva, 2001 (Poland, Finland, Russia)
	23	20 + neo-X$_1$X$_2$Y				
	23	20 + neo-X$_1$X$_2$Y			A large autosome pair*	Nokkala et al., 2006 (Finland)
	23	20 + neo-X$_1$X$_2$Y	(TTAGG)$_n$			Present study (Poland)
Cacopsylla sorbi X(0) cytotype	25	24 + X(0)				Suomalainen & Halkka, 1963: as Psylla (Finland)
Cacopsylla sorbi Neo-XY cytotype	22	20 + neo-XY		A medium-sized autosome pair (AgNOR-banding)*		Maryańska-Nadachowska et al., 1996 (Holand) Maryańska-Nadachowska & Grozeva, 2001 (Finland)
	22	20 + neo-XY				
	22	20 + neo-XY	(TTAGG)$_n$		A large autosome pair*	Present study (Poland)
Cacopsylla sorbi Neo-X$_1$X$_2$Y cytotype	21	18 + neo-X$_1$X$_2$Y				Grozeva & Maryańska-Nadachowska, 1995 (Finland)
Boeopelma foersteri	15**	14 + X(0)		Largest autosome pair (based on a gap)		Suomalainen & Halkka 1963: as *Psylla* (Finland)
	15**	14 + X(0)		–		Nokkala et al., 2000: as *Psylla* (Poland, Finland, Russia, Georgia)
	15**	14 + X(0)		Largest autosome pair (based on a gap)		Nokkala et al., 2004: as *Psylla* (Finland)
	15**	14 + X(0)	(TTAGG)$_n$	Largest autosome pair (AgNOR-banding)	Largest and second largest autosome pairs	Present study (Poland)
Fam. Aphalaridae **Subfam. Rhinocolinae**						
Rhinocola aceris	11	10 + X(0)		–		Maryańska-Nadachowska et al., 1992a (Poland)
	11**	10 + X(0)				Maryańska-Nadachowska, 1999 (Finland, Poland)
	11**	10 + X(0)				Nokkala et al., 2000 (Finland, Russia, Georgia, Poland)
	11***	10 + X(0)	(TTAGG)$_n$		A large autosome pair	Present study (Poland)

* This pair was determined as medium-sized in the cited papers, whereas we suggest that it is one of the large pairs. ** Males in the studied populations were either with or without one to three supernumerary B-chromosomes. *** All studied males carried one B chromosome.

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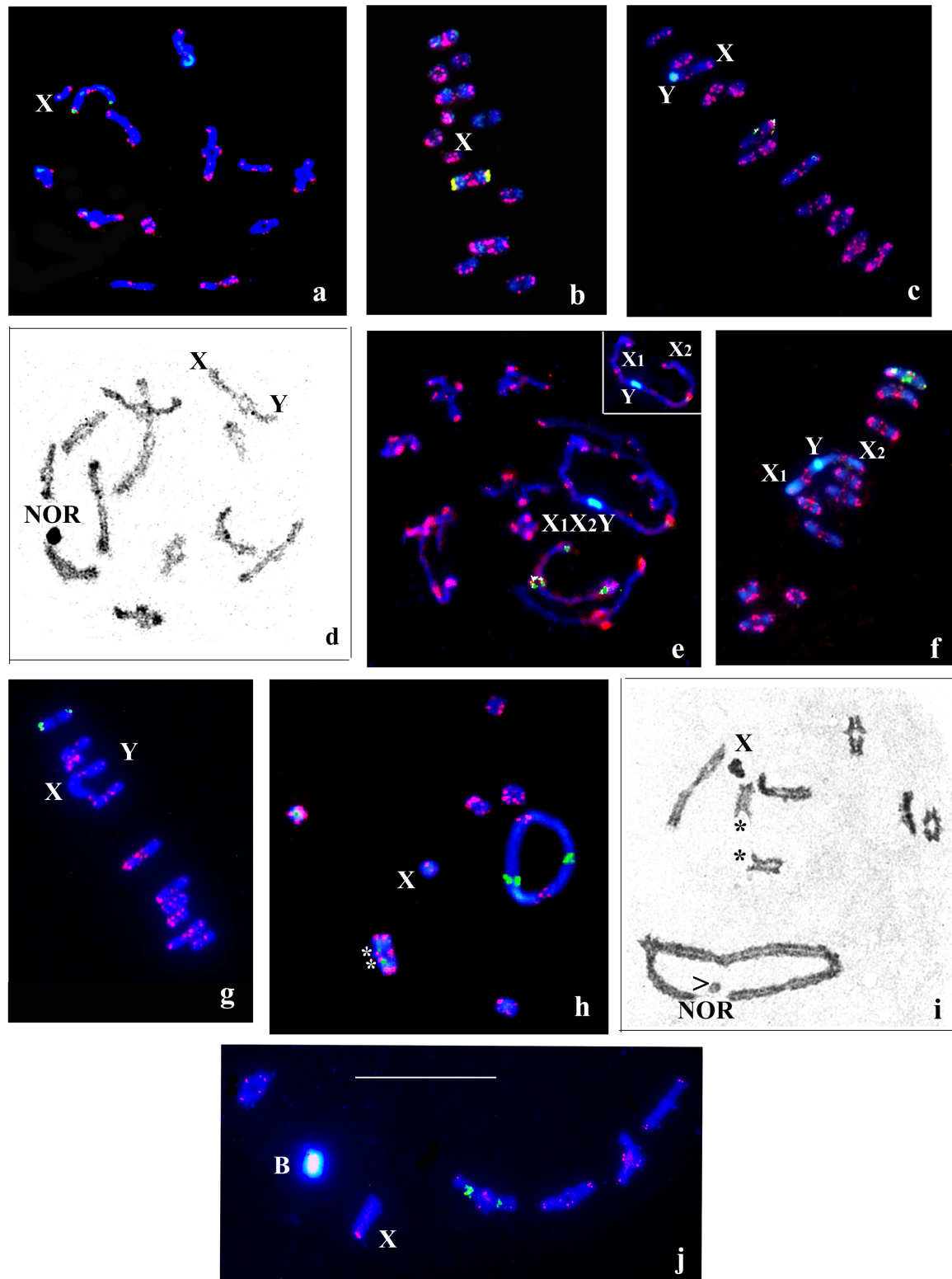
Fig. 1. Examples of FISH with rDNA (green signals) and (TTAGG)$_n$ telomeric (red signals) probes (a–c, e–h, j) and those of AgNOR-banding (d, i) in male meiotic chromosomes of five jumping plant-lice species (Psylloidea). *Psylla alni*, n = 12 + X (a, b): diakinesis/metaphase I (a) and metaphase I/anaphase I (b). rDNA sites are located sub-terminally/terminally on both homologues of a large bivalent; *Cacopsylla mali*, the neo-XY cytotype, 2n = 11 + neo-XY (c, d): metaphase I/anaphase I, rDNA sites are located sub-terminally/terminally on both homologues of a large bivalent, one homologue shows rDNA-site at the chiasmate region of the bivalent while the other homologue shows it on the opposite region of the bivalent, DAPI-positive region is present on the neo-Y (c), diplotene/diakinesis, NOR is located on a large bivalent (d); *C. mali*, the X$_1$X$_2$Y cytotype, n = 10 + neo-X$_1$X$_2$Y (e, f): diplotene/diakinesis, rDNA-sites are located on both ends of every homologue of a large bivalent, DAPI-positive region is seen at least on Y chromosome (e, framed is neo-X$_1$X$_2$Y trivalent), metaphase I, rDNA-sites are located on both ends of every homologue of a large bivalent, DAPI-positive regions are seen on X$_1$ and Y chromosomes (f); *Cacopsylla sorbi*, the XY cytotype, n = 10 + neo-XY: metaphase I/anaphase I, rDNA-sites are located sub-terminally/terminally on both homologues of a large bivalent (g); *Baeopelma foersteri*, n = 7 + X (h, i): diakinesis/metaphase I, rDNA sites are located interstitially on both homologues of the largest bivalent, the second largest bivalent displays sub-terminal/terminal rDNA sites on both homologues as well (asterisks) (h), diakinesis, NOR is located on the largest bivalent (> indicates a nucleolus remnant), the homologues of the second largest bivalent are present as univalents (asterisks) being most probably connected by thin threads with NOR (i); *Rhinocola aceris*, n = 5 + X + B: diakinesis/metaphase I, rDNA sites are located interstitially on both homologues of a large bivalent, B chromosome is DAPI-positive (j). Bar = 10 µm.

Microscopy and imaging

AgNO$_3$-banded slides were analyzed using an Olympus BX 51 light microscope with an Olympus C-35 AD-4 camera. FISH images were taken using a Leica DM 6000 B microscope with a 100× objective, Leica DFC 345 FX camera, and Leica Application Suite 3.7 software with an Image Overlay module (Leica Microsystems, Wetzlar, Germany). The filter sets applied were A, L5 and N21 (Leica Microsystems).

RESULTS

FISH analyses were carried out on a total of 27 males of *P. alni*, *C. mali* (two cytotypes), *C. sorbi*, *B. foersteri*, and *R. aceris*, which differed from each other in chromosome number and sex chromosome system. The first four species belong to the largest family Psyllidae, while the lattermost species to the medium-sized family Aphalaridae. In two species, *C. mali* and *B. foersteri*, the number and location of NORs were additionally examined using the AgNOR method. The basic data obtained in this study, together with chromosome data available in the literature for each of the above species, are summarized in Table 2. Representative images of meiotic karyotypes subjected to AgNOR-banding and FISH with the 18S rDNA and (TTAGG)$_n$ probes are shown in Fig. 1a–j.

Psylla alni

2n = 24 + X (Fig. 1a, b)

Four males and 24 prophase/metaphase I cells were studied, all showing 12 autosomal bivalents and an X-univalent (Fig. 1a, b: n = 12 + X). Approximately three larger bivalents could be distinguished, with the X being one of the small chromosomes of the set. Each bivalent formed one sub-terminal/terminal chiasma. FISH revealed TTAGG telomeric repeats in all plates, the signals were single, double or sometimes numerous on the chromosomes. Moreover, they could be absent on one of the homologues or on one of the ends of a homologue. 18S rDNA-FISH sites were located sub-terminally/terminally on a large bivalent.

Cacopsylla mali

2n = 22 + neo-XY (Fig. 1c, d); 2n = 20 + neo-X1X2Y (Fig. 1e, f) (see explanation in the discussion)

Six males, 18 prophase/metaphase I and 4 metaphase II nuclei were studied. Although all males were collected at the same time from the same *Malus* sp. tree, four of them showed 11 autosomal bivalents and a heteromorphic sex chromosome bivalent (Fig. 1c, d: n = 11 + XY), while the two remaining males had one less autosomal bivalent and a trivalent consisting of three sex chromosomes (Fig. 1e, f: n = 10 + X$_1$X$_2$Y). In the former karyotype (the XY cytotype), the sex chromosome bivalent is one of the largest elements of the set, whereas in the latter (the X$_1$X$_2$Y cytotype), the sex trivalent is notably larger than the sex bivalent in the former. Each autosomal bivalent formed one sub-terminal/terminal chiasma. In both cytotypes, FISH revealed TTAGG repeats on all autosomal bivalents and on the neo-sex chromosomes. Furthermore, the neo-Y chromosome of the neo-XY cytotypes and both the neo-Y and neo-X$_1$ chromosomes of the X$_1$X$_2$Y cytotype were found to carry large DAPI-positive blocks of heterochromatin (Fig. 1c, e, f). 18S rDNA-FISH revealed the presence of rDNA on one of the large bivalents in both cytotypes. In the XY cytotype, the rDNA signals were present on one end of every homologue, whereas in the X$_1$X$_2$Y cytotype both ends of every homologue showed signals of rDNA. Certain bivalents also appeared to carry additional hybridization signals of rRNA genes, which were generally very small and inconstant (not shown). In the XY cytotype, one of the large bivalents, most likely that which showed prominent rDNA-FISH signals, carried NORs as detected by the AgNOR technique (Fig. 1d).

Cacopsylla sorbi

2n = 20 + neo-XY (Fig. 1g) (see explanation in the discussion)

Five males, 12 prophase/metaphase I and 8 metaphase II nuclei were studied. Males showed 10 autosomal bivalents and a heteromorphic sex chromosome bivalent in the first meiotic division (Fig. 1g: n = 10 + XY). Among the autosomal bivalents, at least 6 larger and 4 smaller ones could be distinguished, with the XY pair being the largest element of the karyotype. Each autosomal bivalent formed one sub-terminal/terminal chiasma. FISH revealed the presence of TTAGG telomeric repeats on all chromosomes, however, some bivalents lacked visible signals on separate homologues. While a clear 18S rDNA-FISH signals were located sub-terminally/terminally on one of the large bivalents.

Baeopelma foersteri

2n = 14 + X (Fig. 4h, i)

Seven males, 24 prophase/metaphase I and 3 metaphase II nuclei were studied. Males showed 7 bivalents and a univalent X chromosome in the first meiotic division (Fig. 1h, i: n = 7 + X). One of the bivalents was several times larger than the second largest bivalent of the karyotype. The X chromosome was one of the smallest elements of the set. Each bivalent formed a single sub-terminal/terminal chiasma and only the largest bivalent had two sub-terminal/terminal chiasmata in every nucleus. FISH revealed the presence of TTAGG telomeric repeats on all chromosomes. 18S FISH signals were located interstitially on each homologue of the largest bivalent, with the second largest bivalent also showing rDNA sites. After silver staining, the position of NOR appeared to coincide with that of the rDNA-FISH signals on the largest bivalent (Fig. 1i). The homologues of the second largest bivalent were present as univalents (Fig. 1i), probably being connected by thin threads to another NOR.

Rhinocola aceris

2n = 10 + X (Fig. 1j)

Five males, 30 prophase/metaphase I and 4 metaphase II nuclei were studied. In all males, the karyotype consisted of 5 bivalents and a univalent X chromosome as well as a rather large and heterochromatic B chromosome in the first meiotic division (Fig. 1j: n = 5 + B + X). Each bivalent was observed to form one sub-terminal/terminal chiasma. The

X chromosome is large and most probably exceeds in size the majority of chromosomes in the karyotype. FISH revealed the presence of TTAGG telomeric repeats on chromosomes; however some, including the X, displayed no visible signals on one or both ends. No evident TTAGG signals were present on the B chromosome. 18S rDNA-FISH signals were located interstitially on one of the largest bivalents.

DISCUSSION

Standard karyotypes

The standard karyotypes of species examined in the present study have previously been reported (see Table 2); the data obtained herein coincide with the previous descriptions. In the five species studied we found six different karyotypes which represent almost all of the basic variants currently known in jumping plant-lice species. The male karyotype 2n = 24 + X of *Psylla alni* is known to be a conserved pattern found in most species and is considered to be evolutionarily ancestral for Psylloidea (Maryańska-Nadachowska, 2002). *Cacopsylla mali* and *C. sorbi* are unique within the superfamily in having derived sex chromosome systems originating from one or several X-autosome fusions (Grozeva & Maryańska-Nadachowska, 1995). For *Cacopsylla sorbi*, previous studies described two male karyotypes: 2n = 24 + X in two males examined in a population from Finland (Suomolainen & Halkka, 1963) and 2n = 20 + neo-XY in several European populations, including that from Finland (Grozeva & Maryańska-Nadachowska, 1995; Maryańska-Nadachowska et al., 1996; Maryańska-Nadachowska & Grozeva, 2001). The latter chromosome form (XY cytotype) studied in the present work could have originated from 2n = 24 + X through at least two fusions, one of them between two pairs of ancestral autosomes resulting in 22 + X (not yet found), and another between one of the fusion chromosomes and the X. In *Cacopsylla mali*, a fusion first occurred between an autosomal pair and the X in the progenitor karyotype 2n = 24 + X, resulting in 2n = 22 + neo-XY. A further fusion then occurred between the neo-XY and another autosomal pair resulting in 2n = 20 + neo-X_1X_2Y (Grozeva & Maryańska-Nadachowska, 1995). Remarkably, males with different karyotypes (neo-XY and neo-X_1X_2Y cytotypes) were found to coexist within different geographically distant populations while being morphologically indistinguishable from each other (Grozeva & Maryańska-Nadachowska, 1995; Maryańska-Nadachowska & Grozeva, 2001; Nokkala et al., 2004; present study). One may speculate that males displaying neo-XY and neo-X_1X_2Y systems belong to different coexisting sex chromosome races or even cryptic species; however, such a suggestion is highly improbable. These males are most likely conspecific. A reason for the neo-X_1X_2Y system to be present in a polymorphic state (together with the neo-XY system) in different geographically distant populations of *Cacopsylla mali*, has been attributed to the high frequency of unbalanced gametes produced during meiosis in X_1X_2Y individuals, thus resulting

in the neo-X_1X_2Y system being unable to become fixed in a population (Nokkala et al., 2004).

Baeopelma foersteri and *Rhinocola aceris* had lower than modal chromosome numbers, namely 2n = 14 + X and 2n = 10 + X (male), respectively. In addition, some *R. aceris* populations (including the one studied here) are known to have B chromosomes (reviewed in Maryańska-Nadachowska, 1999). Previous studies of B chromosomes in *R. aceris* have provided interesting insights into their evolutionary importance: incorporation of B chromosome in achiasmate segregation mechanisms with the X chromosome in different populations shows consecutive stages of its conversion into a Y chromosome (Nokkala et al., 2000; Nokkala & Nokkala, 2004). In *B. foersteri*, the presence of an extremely large chromosome pair equivalent in size to some 5 or 6 standard chromosomes (Suomalainen & Halkka, 1963) is certainly an evidence of the chromosome fusions which have occurred in its karyotype evolution. Taking 2n = 25 to be the ancestral karyotype of Psylloidea (Maryańska-Nadachowska, 2002), a total of 5 fusions between autosomal pairs are required to produce the *B. foersteri* karyotype 2n = 15 from a species with 12 pairs of bivalents.

Characterization of karyotypes using FISH with telomeric and 18S rDNA probes

In recent years, FISH has been expanding our understanding of the genetic basis of karyotype evolution in insects with holokinetic chromosomes. Our study represents the first application of this method to the chromosomes of jumping plant-lice. Both the telomeric sequence (TTAGG)$_n$ and ribosomal DNA (18S rDNA probe) were physically mapped in five species differing in chromosome numbers and sex chromosome systems. Telomeres of all studied species were observed to have the (TTAGG)$_n$ motif. This tandemly repeated TG-rich pentanucleotide sequence is known to be generally characteristic (albeit not universal) of Insecta and is considered to be an ancestral DNA motif of the telomeres of insects and also all arthropods (Sahara et al., 1999; Frydrychová & Marec, 2002; Frydrychová et al., 2004; Vítková et al., 2005). Taken together with our present data on Psylloidea, the (TTAGG)$_n$ motif is currently known to be encountered in all major Hemiptera clades, including (in addition to Psylloidea) Coccoidea (Mohan et al., 2011), Aphidoidea (Bizzaro et al., 2000; Frydrychová et al., 2004; Monti et al., 2011; Manicardi et al., 2015), Aleyrodoidea (Frydrychová et al., 2004; Luan et al., 2018), Auchenorrhyncha (Frydrychová et al., 2004; Maryańska-Nadachowska et al., 2013, 2016; Golub et al., 2014; Kuznetsova et al., 2015a, c; Anjos et al., 2016), Coleorrhyncha (Kuznetsova et al., 2015b), and Heteroptera, in which at least all the more basal groups studied to date have this motif (Kuznetsova et al., 2012; Chirino et al., 2017; Angus et al., 2017). These findings strongly support the ancestral nature of the so-called insect telomeric motif (TTAGG)$_n$ in the monophyletic order Hemiptera.

In addition to the canonical DNA sequences located at the terminal ends of chromosomes, non-terminal TTAGG repeats, known as interstitial telomeric sequences (ITSs),

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have been observed in many eukaryotes, from yeasts to humans, and are often considered to be relics of the tandem fusion of ancestral chromosomes during karyotype evolution (reviewed by Lin & Yan, 2008). In insects, ITSs consisting of the (TTAGG)$_n$ motif have so far been identified in only a few species. This includes those with holokinetic chromosomes such as the moth *Orgyia antiqua* (Linnaeus, 1758) (Rego & Marec, 2003) and three species of the giant water bug genus *Belostoma* Latreille, 1807 (Chirino et al., 2017), all with reduced chromosome numbers resulting from autosome fusions. Considering that the telomeres are responsible for chromosome stability and integrity, it can be hypothesized that a prerequisite for the formation of end-to-end chromosome fusions should be either the inactivation or elimination of telomeres (Chirino et al., 2017 and references therein). In support of this, a study by Rego & Marec (2003) found no ITSs on a fused chromosome of a different moth species, *Ephestia kuehniella* (Zeller, 1879), suggesting that the telomeres had probably been lost prior to the fusion of the two original chromosomes. Data obtained in our study also strengthen the aforementioned hypothesis. Both the neo-XY bivalents and neo-X$_1$X$_2$Y trivalents of *Cacopsylla sorbi* and *C. mali* displayed hybridization signals of the (TTAGG)$_n$ probe, clearly demarcating the telomeric regions of chromosomes involved in the neo-sex chromosome systems. However these chromosomes showed no ITSs despite originating from several fusions between autosomes and sex-chromosomes (Grozeva & Maryańska-Nadachowska, 1995). Similarly, no hybridization signals of the telomeric probe were revealed in the interstitial sites of the fused chromosome pair in *B. foersteri* that has a reduced chromosome number (2n = 15). The absence of ITSs may indicate that the telomeric repeats were lost before the original chromosomes fused, as has been suggested for *Ephestia kuehniella* (Rego & Marec, 2003), or that they were inactivated (for other possible explanations for the phenomenon, see Chirino et al., 2017). Moreover, it cannot be excluded that these sequences are in fact present on the fused chromosomes in such a low copy number as to be undetectable by FISH analyses.

The psyllid species examined in our study showed a considerable variation in the number and intensity of the TTAGG signals on almost all chromosomes, these differences also being observed between homologues of a bivalent. Such a variation has been recorded for the giant water bug genus *Belostoma* (Heteroptera) presumably due to ascribed differences in the length of target TTAGG sequences and/or differences in hybridization efficiencies of the FISH experiments performed (Chirino et al., 2017).

In all species (with the exception of *Baeopelma foersteri*) the major rDNA clusters were revealed on a single pair of autosomes. In *Psylla alni*, *Cacopsylla sorbi*, and *C. mali*, the clusters were located sub-terminally/terminally on one of the large pairs of autosomes, and NORs, when studied by the AgNOR method, appeared to be similarly located on a large bivalent in these species (Maryańska-Nadachowska et al., 1992a, b; Maryańska-Nadachowska & Grozeva, 2001; present study). Unfortunately, with no

additional markers it is difficult to speculate whether this pair is the same (homeologous) in different karyotypes and thus whether the species share a syntenic location of their rDNA arrays. It is noteworthy that in the neo-XY cytotype of *C. mali* small additional hybridization signals of rDNA-FISH were present on different chromosomes, thus suggesting that the genome of this species contains an amount of RNA cistrons distributed along various chromosomes. Moreover, only a single NOR, as revealed by silver staining, was transcriptionally active during the preceding interphase (Goodpasture & Bloom, 1975). In contrast to the above three species, both species with a reduced number of chromosomes, *Rhinocola aceris* and *Baeopelma foersteri*, were shown to have interstitially located rDNA clusters; in the former these were housed on one of the largest pairs of autosomes, and for the latter, on both the largest and the second largest pairs of autosomes. FISH results combined with those of silver staining appeared to indicate that it was a NOR-bearing pair of autosomes which had been involved in the fusion events during the evolution of *B. foersteri*.

In conclusion, the present study contributes to knowledge on Psylloidea genomes by reporting for the first time the distribution of rDNA clusters and (TTAGG)$_n$ telomeric sequences in holokinetic chromosomes of five species with different karyotypes. Moreover, the data provide hitherto missing evidence that the insect type (TTAGG)$_n$ telomeric motif is an ancestral trait for all Hemiptera. Future karyotype analyses using FISH should be undertaken on other jumping plant-lice species to gain a more comprehensive view of karyotype evolution of the superfamily Psylloidea.

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