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Cytogenetic characteristic of *Cimex* bed bugs (Heteroptera: Cimicidae)

Cytogenetická charakteristika štěnic rodu *Cimex* (Heteroptera: Cimicidae)

Ph.D. Thesis

Supervisor: Doc. RNDr. Jitka Vilímová, CSc.

Prague, 2020

I hereby declare that the present thesis has not been submitted in order to obtain any academic degree earlier or at another institution. My involvement in the research presented in the thesis is expressed through the authorship order of the included publications and manuscript. All publications and other sources used in the thesis have been cited properly.

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Abstract

The present thesis deals with the phenomenon of additional sex chromosomes in *Cimex lectularius* (Hemiptera: Heteroptera: Cimicidae) using genome size analysis combined with the classical cytogenetic approach. Also, five other cimicid species and 12 species from the family Nabidae were analysed identically for comparative purposes. The thesis also pursues a description of methodical approaches of cytogenetics and flow cytometry in the study of *C. lectularius*.

Recently analysed European specimens of *C. lectularius* from human host exhibited 12 distinct cytotypes, with a variable number of chromosomes X from two to 20 ($2n♂ = 26+X_1X_2Y$ to $26+X_{1-20}+Y$). The fragmentation hypothesis of *C. lectularius* additional chromosomes X origin was established in the second half of the 20th century. However, the present genome size measurements suggest that various chromosomal rearrangements as duplication or deletion besides the fragmentation could occur. Males with basic cytotype $2n = 26+X_1X_2Y$ had average genome size of 2C = 1.94 pg, in contrast male with $2n = 26+X_{1-7}+Y$ yielded 2C = 2.26 pg and also specimens with genome size decrease 2C = 1.69 pg appeared. The most informative turned up to be the relative genome size of sperm cells $n = 13+X_1X_2$ and n = 13+Y, where specimens with higher chromosome number showed relative genome size increase in sperm cells with chromosomes X.

The similar cytogenetic and genome size analysis of the other five cimicid species brought the new record of variability in sex chromosome number of *C. lectularius* from bat hosts and *C. pipistrelli*, $2n = 26+X_1X_2(X_3)Y$ and $2n = 28+X_1X_2(X_3)Y$ respectively. However, in comparison with *C. lectularius* from human, these additional chromosomes X originated mostly by fragmentation and both cytotypes possessed specimens with very similar genome size. Moreover, genome size of all five species analysed was measured for the first time: *C. hemipterus* 2C = 1.47 pg, *C. hirundinis* 2C = 1.61 pg, *C. lectularius* from bats 2C = 1.80 pg, *C. pipistrelli* 2C = 1.68 pg and *Paracimex* cf. *chaeturus* 2C = 1.22 pg.

Genome size analysis in family Nabidae supported the autosomal polyploidization theory, currently sidelined. *Himacerus* species with 2n = 32+XY reached twice as much nuclear DNA content (2C = 9–10 pg) than *Nabis* species with 2n = 16+XY (2C = 4–6 pg). Besides genome size data for all nabid species studied, also the karyotype of *N. biformis*, *N. maoricus* 2n = 16+XY and 2n = 26+XY for *Prostemma aeneicolle* was recorded for the first time.

Abstrakt

Předkládaná dizertační práce představuje návrh vysvětlení původu nadpočetných pohlavních chromosomů štěnice *Cimex lectularius* (Hemiptera: Heteroptera: Cimicidae) pomocí kombinace analýzy velikosti genomu a klasické cytogenetiky. Pro srovnávací účely bylo obdobně analyzováno dalších pět příbuzných druhů z čeledi Cimicidae a 12 druhů z čeledi Nabidae. Práce zahrnuje i popisy metodik zpracování vzorků *C. lectularius*, cytogeneticky a průtokovou cytometrií.

Současné evropské populace *C. lectularius* z lidských hostitelů vykázaly 12 rozdílných cytotypů s rozdílným počtem pohlavních chromosomů X, od dvou do 20 ($2n♂ = 26+X_1X_2Y$ až $26+X_{1-20}+Y$). Hypotéza o původu nadpočetných pohlavních chromosomů X *C. lectularius* pomocí fragmentace byla formulována ve druhé polovině 20. století. Nicméně analýza velikosti genomu naznačuje, že by se mohlo jednat spíše o směs chromosomálních přestaveb typu duplikace či delece, které fragmentaci provázejí. Samci se základním cytotypem $2n = 26+X_1X_2Y$ měli velikost genomu 2C = 1,94 pg, naproti tomu samec s $2n = 26+X_{1-7}+Y$ dosáhl 2C = 2,26 pg, ale objevili se i jedinci se sníženou velikostí genomu 2C = 1,69 pg. Nejdůležitější se však ukázala být relativní velikost genomu spermií $n = 13+X_1X_2$ a n = 13+Y, kdy jedinci s vyšším počtem chromosomů vykazovali navýšení relativní velikosti genomu pouze u spermií nesoucích X chromosomy.

Obdobná analýza chromosomů a velikosti genomu dalších pěti druhů z čeledi Cimicidae přinesla nové záznamy variability počtu pohlavních chromosomů u štěnice *C. lectularius* z netopýřích hostitelů $2n = 26+X_1X_2(X_3)Y$ a *C. pipistrelli* $2n = 28+X_1X_2(X_3)Y$. Avšak v porovnání se situací u *C. lectularius* z člověka tyto nadpočetné chromosomy vznikaly převážně jen fragmentací pohlavních chromosomů X a jedinci z obou cytotypů měli velmi podobné velikosti genomu. Mimo to, velikost genomu všech pěti druhů byla zaznamenána vůbec poprvé: *C. hemipterus* 2C = 1,47 pg, *C. hirundinis* 2C = 1,61 pg, *C. lectularius* z netopýrů 2C = 1,80 pg, *C. pipistrelli* 2C = 1,68 pg and *Paracimex* cf. *chaeturus* 2C = 1,22 pg.

Analýza velikosti genomu zástupců čeledi Nabidae podpořila v současnosti neakceptovanou teorii o autosomové polyploidizaci. Druhy rodu *Himacerus* s 2n = 32+XY dosáhly dvojnásobku jaderné DNA (2C = 9–10 pg) oproti druhům rodu *Nabis* s 2n = 16+XY (2C = 4–6 pg). Vedle velikosti genomu studovaných zástupců čeledi Nabidae, byl poprvé popsán i karyotyp *N. biformis*, *N. maoricus* 2n = 16+XY a 2n = 26+XY pro *Prostemma aeneicolle*.

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1. INTRODUCTION

1.1. Overview of the most important parasitic Heteroptera

The hemipteran suborder Heteroptera (Fig. 1) as one of the largest hemimetabolous taxa consists of more than 42,347 species (Henry 2017). Its astounding diversity is reflected by the ability of heteropteran species to colonise all types of habitats (terrestrial, freshwater, or even the surface of the sea) and also unique sucking mouthparts, common for all Hemiptera, enables Heteroptera to evolve many various life histories (Krenn & Aspöck 2012, Schuh & Weirauch 2020). The most recent common ancestor of Heteroptera used this type of feeding apparatus for predator way of living, but in heteropteran evolution, many feeding strategy shifts occurred. Herbivory originated several times independently and also the hematophagous parasites emerged, however, many mixed feeding transitional strategies exist (Weirauch et al. 2019). For example, accidental blood-feeding in the tribe Cleradini (Pentatomomorpha: Rhyparochromidae). These Heteroptera are able to feed on blood taken from their engorged triatominae prey or even facultatively suck on vertebrate hosts (e.g. Torres et al. 2000).

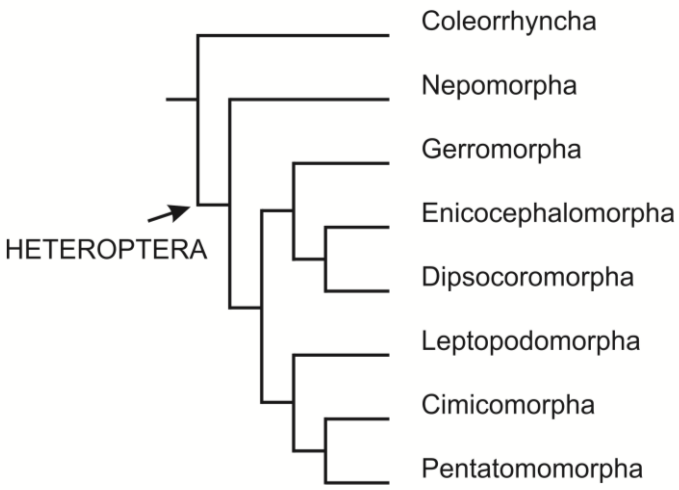

Figure 1. Cladogram of seven Heteroptera infraorders with sister taxa Coleorrhyncha as outgroup according to Weirauch et al. (2019).

But the obligatory parasites from infraorder Cimicomorpha (Fig. 1) are the best known. Family Reduviidae (Fig. 2) with more than 6,878 worldwide species (Henry 2017) comprises mainly predaceous species, but subfamily Triatominae, kissing bugs, represents 140 strictly hematophagous parasitic species, some of them transmit the infamous Chagas disease in South America (e.g. Bardella et al. 2008, Panzera et al. 2010, 2012). Another parasitic cimicomorphan

family is Polyctenidae, bat bugs, 32 species. They are rare viviparous permanent wingless parasites of bats in tropical areas of Old World (subfamily Polycteninae) and New World (subfamily Hesperocteninae) (e.g. Maa 1964, Schuh et al. 2009, Calonge-Camargo & Pérez-Torres 2018). However, the true cimicomorphan "superstar" among obligatory vertebrate blood-feeding insect ectoparasites is the family Cimicidae (Fig. 2) with 110 species in six subfamilies (Henry 2017). *Cimex lectularius* Linnaeus, 1758 from temperate climate zone and tropical *C. hemipterus* Fabricius, 1803, both from subfamily Cimicinae, are the only broadly distributed cimicid species obligatorily connected to human as a host.

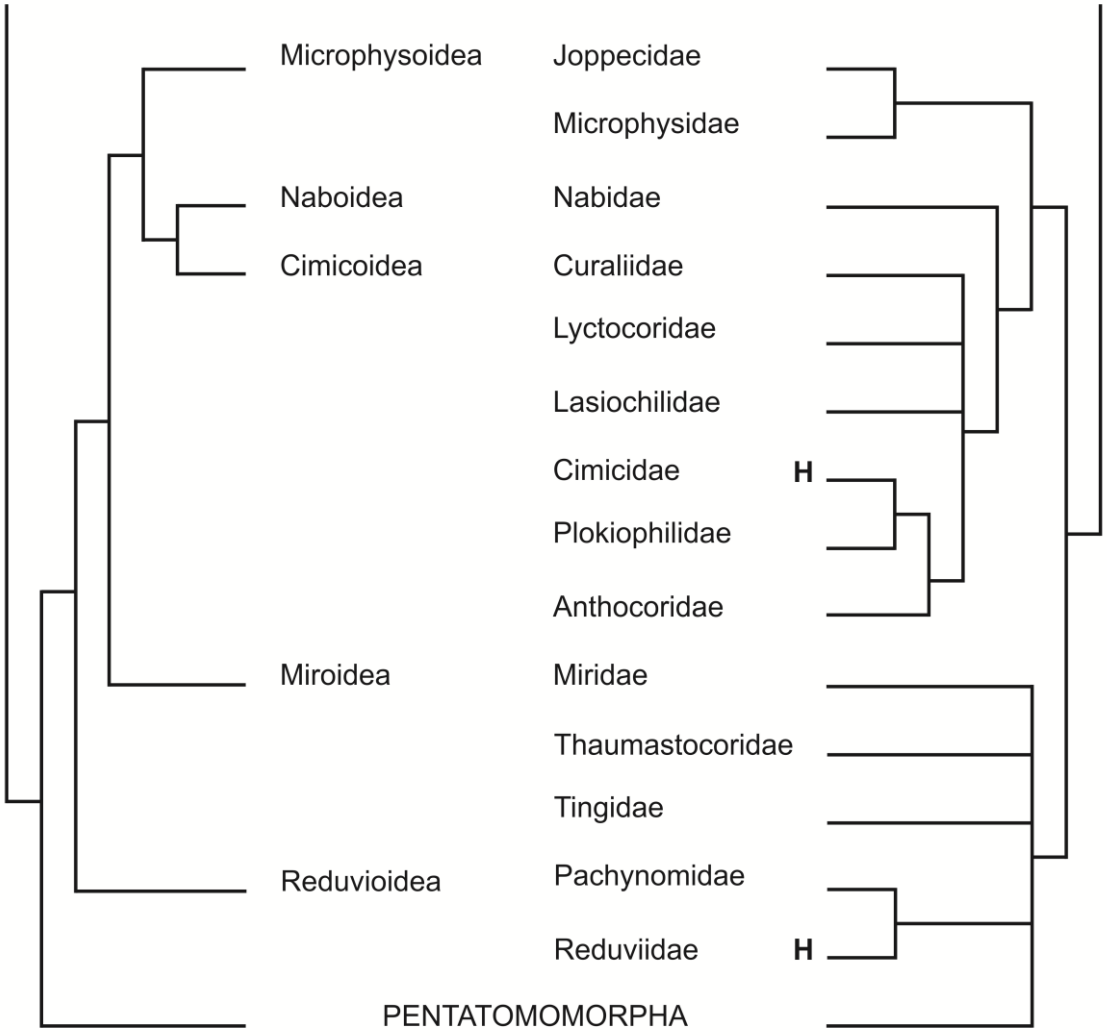

Figure 2. Simplified cladogram of main cimicomorphan superfamilies and families according to Weirauch et al. (2019). H = family includes hematophagous parasitic species.

Cimex lectularius originally parasitized bats and probably shifted to humans in caves, nowadays there exist two strains which parasitize either human or bat as specific hosts. The gene

flow between these two strains is limited and rather asymmetric with direction from human- to bat-parasitizing populations, therefore, strains differ slightly in morphology and also some genetic differences were detected (e.g. Balvín et al. 2012).

It is noteworthy that ancestral cimicids were probably not host generalist (parasitize more taxa), but specialist (parasitize particular genus) (Roth et al. 2019), therefore the hematophagy supposedly did not evolve from facultative blood-feeding of predaceous bugs as in Anthocoridae (Štys 1973, Krinsky 2019) as proposed Weirauch et al. (2019). The opinion, that the original cimicid host were bats (Usinger 1966), were also doubted by Roth et al. (2019) because the fossil of extinct heteropteran family Vetanthocoridae related to Cimicidae (Yao et al. 2006, Engel 2008) dated phylogeny tree of cimicids as more than 30 million years older than oldest known bat fossil (Simmons et al. 2008). However, cimicid species parasitizing humans are considered as generalists, and also shifts of ancestral bed bugs to bat and bird hosts occurred several times (Roth et al. 2019).

Despite the fact, that human parasitizing cimicids have not naturally transmitted any pathogen on humans so far (e.g. Burton 1963, Blow et al. 2001, Doggett 2018), they are a true worldwide scourge, which causes large economic damage in form of expenses for its eradication from human dwellings and psychic trauma to infested dwellings inhabitants (Reinhardt & Siva-Jothy 2007, Doggett et al. 2018). Cimicids were targeted by numerous types of research effort, for example: public health (Hwang et al. 2005), sociology (Reinhardt et al. 2008), behavioral biology and semiochemical analysis (Weeks et al. 2011a, b), population dynamics (Balvín et al. 2012, Zorrilla-Vaca et al. 2015), pyrethroid insecticides resistance (Romero et al. 2007, Lilly et al. 2015), genomics (Benoit et al. 2016), evolution of host switches (Roth et al. 2019) and also cytogenetics (Grozeva et al. 2010, 2011, Sadílek et al. 2013, 2019a, b).

1.2. *Cimex lectularius* cytogenetic features

Cimicidae, as well as all Heteroptera, share a peculiar cytogenetic phenomenon – holokinetic chromosomes. This chromosome type with large/numerous kinetochores covering their surface enables its owners to go by a very different way of karyotype evolution than other species with the single localized kinetochore (centromere) on chromosomes. For example, chromosomal rearrangements like fragmentation and fusion are not necessarily lethal and therefore are quite frequent in organisms with holokinetic chromosomes. Fused chromosomes cannot become dicentric and pieces of fragmented chromosomes also do not have any problems

to connect to the spindle fibres during the division of nucleus, therefore they are not lost (e.g. Mandrioli & Manicardi 2003, Papeschi & Bressa 2006, Schvarzstein et al. 2010).

The very extensive fragmentations were recorded in Cimicidae species, especially in human bed bug *C. lectularius* by Darlington (1939), Slack (1939), and Ueshima (1966). The basic cytotype, the most frequent and with the lowest chromosome number, of *C. lectularius* male is $2n = 26+X_1X_2Y$, however, a unique continuum of specimens with cytotype up to $2n = 26+X_{1-15}Y$ was recorded (Darlington 1939, Slack 1939, Ueshima 1966). The majority of the analysed Heteroptera species did not exhibit variability in sex chromosome number, on the other hand, the sex determination systems with a stable number of multiple (more than two) sex chromosomes occurs across the whole Heteroptera (e.g. Papeschi & Bressa 2006, Kuznetsova et al. 2011).

The additional chromosomes of *C. lectularius* were supposed to be the chromosome X fragments according to the observation of the specific, also called postreductional or inverted (Viera et al. 2009), meiotic division of the sex chromosomes, where the X and Y chromosomes segregate reductionally as late as in the second meiotic division (Ueshima 1966, 1979). Additional chromosomes were also discussed as B chromosomes (Ueshima 1966), however, their behaviour in meiosis, non-random distribution, and isochromatic staining suggest the sex chromosome origin in *C. lectuarius* (Sadílek et al. 2013). Moreover, sometimes they do not even increase the nuclear genome size (Sadílek et al. 2019a).

1.3. Insect genome size analysis

Genome size analysis by flow cytometry method (FCM) is a very frequently used technique in botany, especially for the ploidy determination (e.g. Leitch & Bennet 2004). In zoology, the use of FCM is not so common and it is mostly used only for indicative genome size measurements (e.g. Hanrahan & Johnston 2011, Jacobson et al. 2013, Gregory 2020). However, FCM information value is greatly elevated in combination with cytogenetical research (e.g. Morgan-Richards 2005, Novotná et al. 2011, Sadílek et al. 2019a, b, manuscript).

An interesting pattern of the genome size constrains is observable among all insects analysed so far. Holometabolous insect genome size is pushed down by the necessity of abrupt development during the pupal stage, therefore the maximum is around 2C = 4 pg (2C = genome size of diploid cell, i.e. two sets of chromosomes). In the contrast, hemimetabolous insects can afford, due to its gradual metamorphosis during larval stages, to have a much broader genome

size range, up to 2C = 34 pg, recorded so far (e.g. Gregory 2002, 2020, Hanrahan & Johnston 2011). A similar feature, when species with fast reproduction or short life span possess small genome size is suggested also for annual plants or parasitic insets in general (Gregory 2002, Johnston et al. 2004).

Heteroptera genome size research is quite deficient in comparison with other large insect orders as Coleoptera, Hymenoptera, and Diptera (Gregory 2020). The most explored genome sizes among Heteroptera (Table S1) come from 25 species of family Reduviidae, due to intensive research of South American Triatominae which spread the infamous Chagas disease (Bargues et al. 2006, Panzera et al. 2010). Only very few species, 15 in total, from other families were analysed (Gregory 2020): Belostomatidae (Papeschi 1988, 1991), Cimicidae (Benoit et al. 2016), Miridae (Hanrahan & Johnston 2011, He et al. 2016), Pentatomidae (Hanrahan & Johnston 2011) and Pyrrhocoridae (Bier & Muller 1969, Maddrell et al. 1985). Unfortunately, all genome size studies of 31 Pentatomidae species conducted by Schrader & Hughes-Schrader (1956, 1958) and Hughes-Schrader & Schrader (1956, 1957) (Table S2) content only sample/standard ratio of studied species and unknown standard ("frog" and "mantid"). Such results are therefore incomparable with any other study focusing on genome size.

The overall genome size range of all analysed Heteroptera is the same as in analysed species of family Reduviidae 2C = 0.52–5.8 pg, however, biochemical analysis of Lagowski et al. (1973) reported doubtful genome size of *Oncopeltus fasciatus* (Dallas, 1852) (Lygaeidae) as 2C = 9.3–12.3 pg. Besides Reduviidae only two other families have more than one or two species analysed: Belostomatidae with six species of 2C = 1.06–3.86 pg (Papeschi 1988, 1991) and Miridae with four species of 2C = 0.7–1.8 pg (Hanrahan & Johnston 2011, He et al. 2016).

2. RESEARCH METHODS

2.1. Basic methodical approaches to chromosome study

Two main techniques of chromosome slides preparation can be met in the recent cytogenetic literature: squashing and spreading (also called hot plate spreading). Both techniques have their own pros and cons. The squashing, widely used in the second half of the 20th century (e.g. Sáez 1950, Leston 1957, Piza 1957, Ueshima 1963, Bressa et al. 2003, Poggio et al. 2006, Grozeva et al. 2010, Yang et al. 2012) is very difficult to perform in the field, but analysed specimens could be fixed (3:1 ethanol or methanol: acetic acid) and the own squashing procedure can be conducted later in the laboratory conditions. On the other hand, the newer spreading

method, adapted by Crozier (1968) and Traut (1976), can be performed under the field conditions with a minimum of technical difficulties. The only crucial requirement is the fresh material because fixed cells cannot be spread well. This technique was used for the analysis of chromosomes of various arthropod taxa later at the turn of 20th and 21st century (e.g. Šťáhlavský & Král 2004, Bressa et al. 2009, van't Hof et al. 2011, Forman et al. 2013, Sadílek et al. 2013, 2015, 2019a, b, manuscript, Adilardi et al. 2014, Chirino et al. 2015).

The squashing technique is proceeded usually with fixed specimens and dissected, mostly very fragile, gonads are squashed in a drop of 45% acetic acid by a cover slip. The slip is removed with a razorblade after freezing on solid CO_2 (e.g. Kuznetsova & Maryańska-Nadachowska 2000, Grozeva et al. 2010, Kuznetsova et al. 2015) or in liquid nitrogen (e.g. Bardella et al. 2010). Then slides should be dehydrated in fresh fixative, (which was found as the most tricky step in my test run of this method, insufficiently described in the literature), air-dried and, stained with Giemsa.

Sadílek et al. (2016) described in detail the optimization of the spreading technique for the holokinetic chromosome preparation from the *C. lectularius* and also other related species of cimicids (Sadílek et al. 2019b). This exact approach was suitable also for chromosome analysis of other Heteroptera, for example Nabidae (Sadílek et al. manuscript) or Enicocephalidae (Sadílek unpublished). Chromosome slides of Coleoptera from family Hydrophilidae (Angus et al. manuscript) and *Androctonus* scorpions from family Buthidae (Sadílek et al. 2015) prepared by this approach were also very successful.

2.2. Suitable tissue selection for the chromosome study

The selection of suitable tissue for the chromosome slides preparation is very important and in general, the tissues with the highest mitotic index (abundance of dividing cells) are preferably utilized. In studies of Heteroptera chromosomes, male gonads, where all stages of meiosis can be recorded, are predominantly used. Testes were found also as the best tissue for analysis of human parasitizing *C. lectularius* and other cimicids. Ovaries showed some mitotic chromosome plates as well, but in much lower abundances than in testes (Sadílek et al. 2013, 2016, 2019a, b). Cimicid males of subadult 5th nymph instar regularly showed all various stages of meiosis and mitosis (Sadílek et al. 2016). In contrast to acyclic *C. lectularius* living in human dwellings, predaceous Heteroptera from family Nabidae strictly stick to some reproductive cycle and the nymphs or adults could be captured during the particular season only, temperate zone concerned. Adult females of nabids provided much more mitotic chromosomes in better

condition than adult males, however, all studied 5th instar subadult males from New Zealand showed very abundant chromosomes in meiosis (Sadílek et al. manuscript). It is therefore suggested, that the age of the Nabidae male specimens is a crucial factor to receive high quality chromosomes and the main meiotic spermiogenesis occurs in the 5th instar subadults. The use of ovaries from adult female nabids to get mitotic chromosomes is also highly recommended.

Other tissue types usually used for chromosome analysis are mesentheron (midgut) and eggs/embryos. These tissues processed by spreading technique were successfully used in several studies focused on Heteroptera from families Notonectidae (Angus et al. 2004), Nabidae (Angus et al. 2008), and Corixidae (Waller & Angus et al. 2005). Embryonic tissues were successfully used also for chromosome preparations in studies focused on Coleoptera from the family Hydrophilidae (Angus 1982, Shaarawi & Angus 1991). But no mitotic metaphases were received from cimicid midgut and embryonal tissue slide analysis (Sadílek et al. 2016). This feature was then partially explained by Rost-Roszkowska et al. (2017). In the detailed histological study of *C. lectularius* and *C. pipistrelli* Jenyns, 1839 midgut, nor cell division nor differentiation of regenerative cells was observed. It was therefore suggested, that the proliferation of these "stem" cells, which in cimicids are distributed individually and do not form any regenerative nests or crypts, occur probably only after the beginning of digestive cells degeneration.

2.3. FISH

The very frequently used advanced cytogenetic approach to study large gene clusters position on the chromosomes is the fluorescent in situ hybridization (FISH). The principle of FISH in brief: target gene sequence is multiplied by polymerase chain reaction (PCR) from genomic DNA, fragmented and labelled for example with biotin (also called indirect FISH). The labelled sequence is then attached (hybridized) to the complementary region on the chromosomes and the signal is amplified by the use of more "layers" of an antibody with a fluorescent stain. Chromosomes are at the end counterstained with 4',6-Diamidino-2-Phenylindole (DAPI). The target sequence is visualized as the region on chromosome/s with a different spectrum of excitation than DAPI after the laser beam illumination. Basic and the most commonly used molecular marker in chromosomal studies with FISH is 18S rDNA. It is the part of the large tandem repetition for ribosomal gene 45S RNA (28S + 5.8S + 18S). This marker gene is a very useful tool to study holokinetic chromosomes which are commonly lack of any other traits for differentiation. Variation can be found also among closely relative species: 18S rDNA can be positioned on sex chromosomes only (e.g. Grozeva et al. 2010, Sadílek et al. manuscript), solely

on autosomes or even on a mix of autosomes and X chromosome as in reduviid *Triatoma delpontei* Romana & Abalos, 1947 (e.g. Panzera et al. 2012).

Grozeva et al. (2010) already showed the position of 18S rDNA on X and Y chromosomes of *C. lectularius* with a basic cytotype of 2n = 26+X_1X_2Y. However, our pilot FISH experiments with the use of this gene probe on *C. lectularius* were successful only with basic-cytotyped specimens 2n = 26+X_1X_2Y, and experiments with specimens which possessed additional chromosomes were not with clearly positive results. Signal was not definitely localized on additional chromosomes and it was rather scattered around them (Sadílek unpublished), thus we decided to investigate the phenomenon of *C. lectularius* additional chromosomes with a different methodical approach. The combination of classic cytogenetics and FCM. In spite of unclear FISH results in *C. lectularius* with additional chromosomes, studied Nabidae (Sadílek et al. manuscript) and Buthidae scorpions (Sadílek et al. 2015) exhibited clearly localised 18S rDNA signals on their chromosomes.

2.4. FCM tissue selection

Authors of recent insect FCM studies mentioned that they used for analysis mainly neural tissues (e.g. Johnston et al. 2004, Hanrahan & Johnston 2011, Benoit et al. 2016). But in fact, they used a whole head capsule which comprises many more tissue types, which could make the analysis much worse (higher coefficient of variation) as described in Hanrahan & Johnston (2011). Within the study of *C. lectularius* genome size (Sadílek et al. 2019a), also the suitability of specific tissues or entire body parts for genome size measurements were tested – gonoducts with sperm cells, eggs/embryos, whole gut, fat body, and head/thorax/abdomen respectively.

The analysis of different tissues can reveal also cells with different ploidy (e.g. Johnston et al. 2004) as 8C (octaploid) cell peaks in samples of *C. lectularius* abdominal tissue and gonoduct with sperm or 1C (haploid) cells in direct sperm cell analysis (Sadílek et al. 2019a). Sperm cells and the mixture of several embryos in eggs (diploid cells) analyses also exhibit clearly observable “male/female” double peaks and therefore the distinct DNA content differences of *C. lectularius* sex chromosomes (Sadílek et al. 2019a). The larger difference between particular peaks of a double peak, the higher number of additional sex chromosomes presented.

All tissues/body parts tested provided 2C (diploid) genome size and the only exception were gut tissue samples, they showed no results and therefore were evaluated as unsuitable for

FCM analysis of *C. lectularius* (Sadílek et al. 2019a). Another highly interesting peculiarity was the impossibility to stain *C. lectularius* sperm cells with propidium iodide (PI), while all other tissues/body parts showed peaks after either DAPI or PI staining. Sperm cells showed double peaks only with DAPI stain, therefore only relative genome size could be measured in that case, due to DAPI stain AT basis affinity.

2.5. FCM sample treatment

The basic procedure of FCM insect sample preparation was adopted from plant sample preparation (e.g. Doležel & Bartoš 2005, Doležel et al. 2007) and a similar procedure was used also for genome study of aphids (Novotná et al. 2011), tree wetas (Morgan-Richards 2005) and spittlebugs (Rodrigues et al. 2016). Sadílek et al. (2019a) performed several tests to select a suitable standard from several vertebrate, insect, and plant model organisms. The optimal standard genome size should not be larger than 2-fold from the studied sample (Suda & Leitch 2010). Genome size around 2C = 7 pg of human and *Xenopus laevis* (Daudin, 1802) was too large, *Drosophila melanogaster* Meigen, 1830 genome size of 2C = 0.36 pg was too small and *Pyrrhocoris apterus* (Linnaeus, 1758) genome size of 2C = 2.44 pg was too similar (Gregory 2020) to genome size of studied *C. lectularius*. As the result, plant *Solanum pseudocapsicum* L. with 2C = 2.61 pg (Temsch et al. 2010) was selected as the primary standard for *C. lectularius* and other cimicids (Sadílek et al. 2019a, b) genome size analyses, as the secondary standard was selected *Bellis perennis* L. 2C = 3.51 pg (Schönswetter et al. 2007). Larger genome sizes in nabid species (Sadílek et al. manuscript) were measured primarily with *B. perennis* and *S. pseucapsicum* was used as a secondary standard in measurements of *Nabis maoricus* Walker, 1873 due to overlapping of sample and primary standard peaks in FCM analysis.

The next test was the stability of genome size of samples at –20°C with increasing time (Sadílek et al. 2019a). PI stained samples showed a 2% decrease of genome size and DAPI 4.7% after 42 days at –20°C. But repeatedly melted samples measurability decreased significantly and only 50% of samples were measurable after three defrosting events. However, samples of some specimens were clearly measurable even after 1,078 days at –20°C. Therefore, the uninterrupted storing of tissue samples at –20°C was suggested as a suitable way of storage and transport as reported by Hanrahan & Johnston (2011). Samples fixed by ethanol or air-dried showed in FCM analysis no results, but Panzera et al. (2004) successfully analysed also tissues fixed in ethanol: acetic acid (3:1).

Also dividing each sample into two parts and staining one with DAPI and the second with PI was highly recommended (Sadílek et al. 2019a) because the different affinity of both stains reveals the AT/GC ratio of the basis contained in the genome, the GC content (e.g. Šmarda et al. 2008, Sadílek et al. 2019 a, b, manuscript). DAPI stain binds preferentially to the surface of AT-rich regions of DNA and expresses relative genome size, but the PI stain intercalates into DNA without any preferences and therefore express the true genome size (e.g. Doležel & Bartoš 2005, Kolář et al. 2013). The GC content could be, besides genome size, an additional FCM feature determining species, which is clearly demonstrated in the case of *Himacerus apterus* (Fabricius, 1798) and *H. mirmicoides* (O. Costa, 1834) (Sadílek et al. manuscript). In these very closely related species is the pattern of GC content completely different, sample/standard ratio of *H. apterus* stained with DAPI and PI yielded a large difference (3.12 and 2.80), but in *H. mirmicoides* were DAPI and PI sample/standard ratio values almost the same (2.60 and 2.58).

3. CYTOGENETICS AND CYTOMETRIC RESULTS

3.1. Distribution of *Cimex lectularius* cytotypes today and in the past

After a rapid decrease of *C. lectularius* numbers by the massive use of DDT in the middle of the 20th century the bed bugs started to re-emerge in all developed countries of the temperate climate zone together with the increasing mobility of their hosts at the end of the 90s (Reinhardt & Siva-Jothy 2007). Sadílek et al. (2013), based on data from the Master degree´s thesis of Sadílek (2012) and extended with two cytotypes of 2n = 2n = 26+X_{1-9}Y and 2n = 26+X_{1-13}Y, published the list of analysed cytotypes within the randomly collected bedbugs of central Europe. This screening showed the present situation of 21st century *C. lectularius* cytotypes distribution in this region compared to findings of Darlington (1939) and Slack (1939) in Great Britain before the huge bottleneck caused by DDT use.

Sadílek et al. (2013) distinguished 12 cytotypes from 2n = 26+X_1X_2Y to 26+X_{1-10}Y, 26+X_{1-13}Y, 26+X_{1-15}Y, and 26+X_{1-20}+Y chromosomes within 116 specimens from 61 localities and showed very similar findings as in studies of Darlington (1939) and Slack (1939) conducted 74 years ago. The range of cytotype variability remained almost unchanged, but the percentage of individuals within different cytotypes in samples shifted distinctly. Darlington (1939) recorded just a few individuals with basic 2n = 26+X_1X_2Y chromosomes and most of his analysed specimens (from seven populations only) possessed derived cytotype with more chromosomes up to 2n = 26+X_{1-14}Y, moreover, Slack (1939) in his three populations, with up to

2n = 26+X_{1-15}Y, recorded no specimen with the basic chromosome number. In contrast Sadílek et al. (2013) presented 44% of analysed specimens with basic 2n = 26+X_1X_2Y, 20% with 2n = 26+$X_1X_2X_3$Y and 11% with 2n = 26+X_{1-4}Y, other cytotypes were represented with one to five specimens, 0.9–4% respectively, i.e., the higher number of chromosomes in cytotype, the fewer specimens recorded. All populations (except one) presented by studies of Darlington (1939) and Slack (1939) also exhibited specimens from several cytotypes, but Sadílek et al. (2013) recorded mostly populations where all specimens possessed the same cytotype – only 25% of populations provided more cytotype variants. Sadílek et al. (2013) also recorded the single *C. lectularius* male specimen with extreme cytotype 2n = 26+X_{1-20}Y (the highest number of X chromosomes among Cimicidae or even all Heteroptera known so far) from a population with mixed cytotypes of high chromosome numbers 2n = 26+X_{1-8}Y, 26+X_{1-15}Y and 26+X_{1-20}+Y. It is not a big surprise that the distribution of *C. lectularius* cytotypes appears random, and did not show any consistent geographic or phylogenetic pattern, due to the way of dispersion of these parasites (Sadílek et al. 2013).

3.2. Chromosome variability in other cimicids

In the family Cimicidae were cytogenetically analysed 53 species with diverse variants of XX/XY sex chromosome system based on differences of X chromosome count. Three species possess a constant basic XY system, four species possess three X chromosomes ($X_1X_2X_3$Y) and two species four X chromosomes (X_{1-4}Y), but the majority (44 species) of cimicids analysed possess two X chromosomes (X_1X_2Y) (summary in Ueshima 1979, Kuznetsova et al. 2011, Sadílek et al. 2016). Interestingly, only the group with X_1X_2Y sex determination includes species where the variable number of chromosome X was recorded.

Studies presented in this thesis recorded additional chromosomes in three cimicid species or strains respectively: *C. lectularius* from human 2n = 26+X_1X_2Y to 26+X_{1-20}Y (Sadílek et al. 2013, 2019a), *C. lectularius* from bats 2n = 26+X_1X_2Y or 26+$X_1X_2X_3$Y and *C. pipistrelli* 2n = 28+X_1X_2Y to 28+X_{1-4}Y (Sadílek et al. 2013, 2019b). *Paracimex* cf. *chaeturus* Ueshima, 1968 showed a high chromosome number of 2n = 41, however, it was not possible to determine karyotype precisely. On the other hand, all so far analysed species of *Paracimex* Kiritshenko, 1913 possess 36 autosomes, therefore estimated karyotype could be with multiple X chromosomes (2n = 36+X_{1-4}Y) similarly as *P. borneensis* Usinger, 1959 (2n = 36+X_1X_2Y and 36+X_{5-9}Y) or *P. capitatus* Usinger, 1966 (2n = 36+X_{2-6}Y) (Ueshima 1968, 1979). Other cimicid

species studied in Sadílek et al. (2019b) – *C. hemipterus* and *C. hirundinis* Lamarck, 1816 showed only a basic karyotype of 2n = 28+X_1X_2Y without any variability.

3.3. Genome size of cimicid cytotypes

FCM technique was found as a very efficient tool for chromosome number variability research in *C. lectularius* (Sadílek et al. 2019a). The genome size analysis in various *C. lectularius* cytotypes (especially with more chromosomes than basic 2n = 26+X_1X_2Y) indicated the mix of additive and deleterious chromosome rearrangements besides sole chromosome fragmentation. Various cytotypes showed specimens with a distinct decrease or increase of nuclear DNA content together with specimens of average DNA content. If only fragmentation would be present, as supposed in former studies (e.g. Ueshima 1966, Grozeva et al. 2010), the genome size would be in all cytotypes the same or at least very similar regardless of the number of chromosomes.

The presence of supernumerary B chromosomes as additional chromosomes would be indicated by a consecutive increase of DNA content in each cytotype with more chromosomes (Gregory 2002, Poggio et al 1998), which was not observed (Sadílek et al. 2019a). Therefore, it seems that this hypothesis of additional chromosome origin in *C. lectularius* was finally falsified.

Sadílek et al. (2019a) also verified that the additional chromosomes are most probably really the sex chromosomes through the FCM analysis of *C. lectularius* sperm cells. Direct analysis of haploid cells reflected very fine differences in relative DNA content (DAPI- stained), which clearly presented an increase of the relative nuclear DNA content of sperm cells bearing X chromosomes together with an increase of chromosome number of specimens. Whereas the sperm cells bearing the Y chromosome demonstrated the stable relative nuclear DNA content in all cytotypes analysed.

Sadílek et al. (2019b) presented the genome size of five cimicid species and compared the results with human bed bug *C. lectularius* from Sadílek et al. (2019a). Average diploid genome size value for males with the basic karyotype of all cimicid species studied: *C. lectularius* from human 2C = 1.94 pg, *C. lectularius* from bats 2C = 1.80 pg, *C. hemipterus* from human 2C = 1.47 pg, *C. pipistrelli* from bats 2C = 1.68 pg, *C. hirundinis* from swallows 2C = 1.60 pg and *Paracimex* cf. *chaeturus* from swifts 2C = 1.20 pg. A peculiar finding is the different pattern of additional sex chromosome origin. In human parasitizing *C. lectularius* prevailed additive or deleterious rearrangements, but in other species with additional chromosomes presented (*C.*

lectularius from bats and *C. pipistrelli*) are probably dominant sole fragmentation events (Sadílek et al. 2019b).

3.4. Comparative research of the family Nabidae

Family Nabidae with 386 species (Henry 2017) was selected as closely related to the cimicids, Naboidea is the sister taxon to Cimicoidea (Fig. 2) (Weirauch et al. 2019), to compare cytogenetic features and genome size. There are two main studied groups within European nabids with distinct chromosome number, *Nabis* Latreille, 1802 with 2n = 16+XY and *Himacerus* Wolff, 1811 with 2n = 32+XY (with data for 22 and four species respectively) (Kuznetsova & Maryańska-Nadachowska 2000, Kuznetsova et al. 2011, Sadílek et al. manuscript). In contrast to cimicid species, where the intraspecific variability is conducted by a different number of sex chromosomes (e.g. Sadílek et al. 2013, 2019a, b), the source of nabid variability is mostly proposed as autosomal fragmentation or B chromosomes (Grozeva & Nokkala 2003, Kuznetsova et al. 2011). However, Sadílek et al. (manuscript) recorded also a single *N. rugosus* (Linnaeus, 1758) male with stable additional sex chromosome in all cells 2n = 19.

A very interesting feature showed the FISH analyses with the 18S rDNA probe (Sadílek et al. manuscript). In *C. lectularius* were recorded two signals, on the X and Y chromosome (Grozeva et al. 2010), the very same pattern as in Nabidae species (Grozeva et al. 2019, Sadílek et al. manuscript). But Nabidae showed also species with the doubled signal on X (*N. biformis* (Bergroth, 1927)) or Y (*N. pseudoferus* Remane, 1949 and *N. rugosus*) chromosome, moreover, the 18S rDNA signal was completely absent on the Y chromosome in *N. maoricus* (Sadílek et al. manuscript). It seems that the 18S rDNA position on nabid sex chromosomes is quite variable and also can be presented even on autosomes as in *Arachnocoris trinitatus* (Bergroth, 1916), where the NOR (nucleolus organiser region, traditionally indicated by silver nitrate staining – colocalise with 18S rDNA signal from FISH) was detected on the largest pair of autosomes (Kuznetsova et al. 2007, Kuznetsova & Grozeva 2008).

Very peculiar data was obtained also from FCM analyses of *Nabis* and *Himacerus* species (Sadílek et al. manuscript) which suggested the solution between two competing hypotheses. The first hypothesis considers the karyotype of 2n = 32+XY as derived from ancestral 2n = 16+XY by polyploidy of whole autosomal set (Leston 1957, Ueshima 1979, Thomas 1996, Kuznetsova & Maryańska-Nadachowska 2000), however, the second hypothesis suggests karyotype of 2n = 32+XY as ancestral and 2n = 16+XY as derived by fusions of all autosomes (Kuznetsova et al.

2004, 2007, Grozeva et al. 2005, Nokkala et al. 2007). Recently Sadílek et al. (manuscript) presented two *Himacerus* species (2n = 32–36+XY) with twice as large genome size as 2C = 9–10 pg in contrast to five *Nabis* species (2n = 16+XY) which showed only 2C = 4–6 pg. Therefore, the autosomal polyploidy event was suggested as the most probable tool of differentiation in the karyotype evolution of these two genera of Nabidae.

3.5. Comparative research of the *Androctonus* scorpions

Holokinetic chromosomes were described in many species of arachnids, insects, nematodes, and plants (Melters et al. 2012), and due to its vast dispersion among different taxa it is suggested their independent origin up to 19 times (e.g. Ubinski et al. 2018). From insects, the Heteroptera is the typical taxon possessing the holokinetic chromosomes and from arachnids are typical scorpions from the family Buthidae. The different origin event of holokinetic chromosomes of these taxa caused also the different way of their karyotype evolution.

Scorpions of basal and the most diversified family Buthidae with 1,214 species (Rein 2020) are cytogenetically unique by the possession of holokinetic chromosomes within the whole order Scorpiones - other families have monocentric chromosomes (e.g. Melters et al. 2012, Sadílek et al. 2015, Ubinski et al. 2018). They exhibit a completely different type of chromosome number variation in comparison to holokinetic chromosomes of Cimicidae or Nabidae. In mentioned Heteroptera very probably dominate fragmentation and duplication resulting in intraspecific chromosome number polymorphism (Sadílek et al. 2019a, b, manuscript) but in studied buthid scorpions of genus *Androctonus* Ehrenberg, 1828 (also in other buthid scorpions) chromosome fissions and fusions resulted in heterozygous translocation and assembly of multivalents, which can be quite extensive (e.g. Moustafa et al. 2005, Sadílek et al. 2015, Almeida et al. 2019). Intraspecific variability of chromosomes in Cimicidae species is exclusively through variation of additional sex chromosomes (Sadílek et al. 2013, 2019a, b), in closely related Nabidae are additional chromosomes mainly autosomes and occasionally are involved also sex chromosome rearrangements (Sadílek et al. manuscript). However, in Scorpiones sex chromosomes are not well morphologically differentiated from autosomes and therefore cannot be distinguished easily (e.g. Mattos et al. 2013, Sadílek et al. 2015), although, Adilardi et al. (2016) suggested the scorpion sex determination system with homomorphic cryptic XY/XX. In *Androctonus* scorpions was found very stable genome organisation, i.e. rigid chromosome number of 2n = 24, on the other hand, many other buthid genera show vast intraspecific variability (Schneider et al. 2009), for example, species of genera *Gint* Kovařík,

Lowe, Plíšková & Šťáhlavský, 2013 with 2n = 18–45, *Hottentotta* Birula, 1908 with 2n = 14–28, *Parabuthus* Pocock, 1890 with 2n = 16–36 and *Tityus* C.L.Koch, 1836 with 2n = 5–32 (e.g. Kovařík et al. 2018, 2019, Mattos et al. 2018, Rein 2020).

4. CONCLUSIONS

- 12 cytotypes from 2n = 26+X_1X_2Y to 26+X_{1-20}+Y were recorded within recent European *Cimex lectularius* strain from a human host
- Intraspecific variability in the number of chromosomes X in *C. lectularius* from bats and *C. pistrelli* was revealed, 2n = 26+$X_1X_2(X_3)Y$ and 2n = 28+$X_1X_2(X_3)Y$ respectively
- Genome size for six species/strains of Cimicidae (males) was stated: *C. hemipterus* 2C = 1.47 pg, *C. hirundinis* 2C = 1.61 pg, *C. lectularius* from bats 2C = 1.80 pg, *C. lectularius* from human 2C = 1.94 pg, *C. pipistrelli* 2C = 1.68 pg and *Paracimex* cf. *chaeturus* 2C = 1.22 pg
- Genome size measurements revealed the most probably chromosomal rearrangements (duplication and deletion) besides plain fragmentation in *C. lectularius* from human, however, *C. lectularius* from bats and *C. pistrelli* showed fragmentation as the most common rearrangement
- Karyotype of 2n = 16+XY for *Nabis biformis* and *N. maoricus* and 2n = 26+XY for *Prostemma aeneicolle* was revealed
- Origin of *Himacerus* species karyotype through autosomal polyploidy from ancestral karyotype presented in *Nabis* species was suggested and genome size for 12 nabid species (males) was stated: *Himacerus* species 2C = 9–10 pg, *Nabis* species 2C = 4–6 pg, *Prostemma* species 2C = 7–8 pg
- FISH did not detect 18S rDNA signal on additional sex chromosomes in *C. lectularius*, but in Nabidae this method revealed species-specific pattern with two, one, or zero 18S rDNA signals on sex chromosomes.
- Various methodical aspects of *C. lectularius* chromosome slide preparation, as well as a methodical approach to genome size measurements, were stated

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6. SUPPLEMENT

Supplementary table 1. The list of genome sizes of all Heteroptera studied so far. Template list excerpted from an online genome size database (Gregory 2020) and completed by data from several more original articles which are missing in the database (reference with asterisk).

Method: BCA = biochemical analysis, FCM = flow cytometry, FD = Feulgen densitometry.

Cell type: BR = brain, S = sperm, TR = trachea, TS = testes, WB = whole body/body parts.

Standard: AC = *Allium cepa*, BeP = *Bellis perennis*, BP = *Bufo paracnemis*, DM = *Drosophila melanogaster*, DV = *Drosophila virilis*, GD = *Gallus domesticus*, HS = *Hommo sapiens*, SP = *Solanum pseudocapsicum*.

1C = genome size of one set of chromosomes (haploid), 2n = diploid chromosome number, - = details were not given by original authors

Taxon	1C (pg)	Sex	2n	Method	Cell type	Standard	Standard 1C (pg)	Reference
Infraorder NEPOMORPHA								
BELOSTOMATIDAE								
Belostoma *bifoveolatum*	1.21	M	29	FD	TS	GD	1.25	*Papeshi 1991
B. dentatum	1.93	M	29	FD	TS	GD	1.25	*Papeshi 1991
B. elegans	1.55	M	29	FD	TS	GD	1.25	*Papeschi 1988
B. elegans	1.46	M	29	FD	TS	GD	1.25	*Papeshi 1991
B. martini	1.11	M	29	FD	TS	GD	1.25	*Papeshi 1991
B. micantulum	0.88	M	16	FD	TS	GD	1.25	*Papeschi 1988
B. oxyurum	0.53	M	8	FD	TS	GD	1.25	*Papeschi 1988
Infraorder CIMICOMORPHA								
REDUVIIDAE								
Dipetalogaster *maximus*	1.29	M	22	FCM	TS	HS	3.50	Panzera et al. 2007
Eratyrus *cuspidatus*	1.26	M	23	FCM	TS	HS	3.50	Panzera et al. 2007
Panstrongylus *geniculatus*	1.42	M	23	FCM	TS	HS	3.50	Panzera et al. 2007
P. herreri	0.61	M	23	FD	TS	BP	3.79	*Schreiber et al. 1972
P. megistus	0.59	M	21	FD	TS	BP	3.79	*Schreiber et al. 1972
P. rufotuberculatus	1.44	M	23	FCM	TS	HS	3.50	Panzera et al. 2007
Rhodnius *colombiensis*	0.58	M	22	FCM	TS	HS	3.50	Diaz et al. 2014
R. ecuadoriensis	0.72	M	22	FCM	TS	HS	3.50	Panzera et al. 2007

Taxon	1C (pg)	Sex	2n	Method	Cell type	Standard	Standard 1C (pg)	Reference
R. pallescens	0.73	M	22	FCM	TS	HS	3.50	Gómez-Palacio et al. 2012
R. prolixus	0.86	-	22	FD	TR	GD	1.15	*Maddrell et al. 1985
R. prolixus	0.26	-	22	-	-	-	-	Petitpierre 1996
R. prolixus	0.75	M	22	FCM	TS	HS	3.50	Panzera et al. 2007
R. prolixus	0.59	-	22	FD	S	DM	0.18	Gregory (unpub. data)
***Triatoma** barberi*	1.23	M	23	FCM	TS	HS	3.50	Panzera et al. 2007
T. brasiliensis	1.09	M	22	FCM	TS	HS	3.50	Panzera et al. 2007
T. delpontei	1.80	M	22	FD	-	AC	16.50	Panzera et al. 1995
T. delpontei	2.90	M	22	FCM	TS	HS	3.50	Bargues et al. 2006
T. dimidiata	0.92–1.07	M	23	FCM	TS	HS	3.50	Panzera et al. 2006
T. infestans	0.82	M	22	FD	TS	BP	3.79	*Schreiber et al. 1972
T. infestans	1.03	M	22	FD	-	AC	16.50	Panzera et al. 1995
T. infestans	1.70	M	22	FCM	TS	HS	3.50	Panzera et al. 2004
T. infestans	1.44–1.98	M	22	FCM	TS	HS	3.50	Bargues et al. 2006
T. longipennis	0.91	M	23	FCM	TS	HS	3.50	Panzera et al. 2007
T. maculata	1.09	M	22	FCM	TS	HS	3.50	Panzera et al. 2007
T. melanosoma	1.53	M	22	FCM	TS	HS	3.50	Bargues et al. 2006
T. nitida	1.35	M	21	FCM	TS	HS	3.50	Panzera et al. 2007
T. platensis	0.70	M	22	FD	TS	BP	3.79	*Schreiber et al. 1972
T. platensis	0.87	M	22	FD	-	AC	16.50	Panzera et al. 1995
T. platensis	1.33	M	22	FCM	TS	HS	3.50	Bargues et al. 2006
T. pseudomaculata	0.57	M	22	FD	TS	BP	3.79	*Schreiber et al. 1972
T. pseudomaculata	1.13	M	22	FCM	TS	HS	3.50	Panzera et al. 2007
T. rubrovaria	0.54	M	22	FD	-	AC	16.50	Panzera et al. 1995
T. rubrovaria	1.17	M	22	FCM	TS	HS	3.50	Bargues et al. 2006
T. ryckmani	1.10	M	23	FCM	TS	HS	3.50	Panzera et al. 2007
T. sordida	1.12	M	22	FCM	TS	HS	3.50	Bargues et al. 2006
T. vitticeps	0.89	M	24	FD	TS	BP	3.79	*Schreiber et al. 1972
NABIDAE								
***Himacerus** apterus*	4.96	F	38	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
H. apterus	4.86	M	38	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
H. mirmicoides	4.54	M	34	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
***Nabis** ferus*	2.40	F	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript

Taxon	1C (pg)	Sex	2n	Method	Cell type	Standard	Standard 1C (pg)	Reference
N. ferus	2.24	M	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
N. maoricus	2.08	F	18	FCM	WB	SP	1.31	*Sadílek et al. manuscript
N. maoricus	1.87	M	18	FCM	WB	SP	1.31	*Sadílek et al. manuscript
N. pseudoferus	2.94	F	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
N. pseudoferus	2.67	M	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
N. punctatus	2.65	F	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
N. punctatus	2.43	M	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
N. rugosus	3.18	F	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
N. rugosus	2.97	M	18	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
Prostemma *guttula*	3.78	F	28	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
P. guttula	3.57	M	28	FCM	WB	BeP	1.76	*Sadílek et al. manuscript
CIMICIDAE								
Cimex *hemipterus*	0.78	F	32	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. hemipterus	0.74	M	31	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. hirundinis	0.86	F	32	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. hirundinis	0.80	M	31	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. lectularius- human host	0.88	F	30	FCM	BR	DV	0.34	*Benoit et al. 2016
C. lectularius- human host	0.84	M	29	FCM	BR	DV	0.34	*Benoit et al. 2016
C. lectularius- human host	0.98	F	30	FCM	WB	SP	1.31	*Sadílek et al. 2019a
C. lectularius- human host	0.97	M	29	FCM	WB	SP	1.31	*Sadílek et al. 2019a
C. lectularius- bat host	0.90	F	30	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. lectularius- bat host	0.90	M	29	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. pipistrelli	0.78	F	32	FCM	WB	SP	1.31	*Sadílek et al. 2019b
C. pipistrelli	0.84	M	31	FCM	WB	SP	1.31	*Sadílek et al. 2019b
Paracimex *cf. chaeturus*	0.60	M	41	FCM	WB	SP	1.31	*Sadílek et al. 2019b
MIRIDAE								
Apolygus *lucorum*	0.90	M	-	FCM	BR	DM	0.18	He et al. 2016
Cyrtorhinus *lividipennis*	0.36	F	-	FCM	BR	DM	0.18	He et al. 2016
C. lividipennis	0.35	M	-	FCM	BR	DM	0.18	*He et al. 2016
Pseudatomoscelis *seriatus*	0.61	F	-	FCM	BR	DV	0.34	*Hanrahan & Johnston 2011
P. seriatus	0.57	M	-	FCM	BR	DV	0.34	*Hanrahan & Johnston 2011
Tytthus chinensis	0.41	F	-	FCM	BR	DM	0.18	He et al. 2016

Taxon	1C (pg)	Sex	2n	Method	Cell type	Standard	Standard 1C (pg)	Reference
Infraorder PENTATOMOMORPHA								
PENTATOMIDAE								
Brochymena *cariosa*	1.26	-	-	FCM	BR	DM	0.18	Hanrahan & Johnston 2011
PYRRHOCORIDAE								
Dysdercus *fasciatus*	1.40	M	16	FD	TR	GD	1.15	*Maddrell et al. 1985
Pyrrhocoris *apterus*	1.22	-	23	FD	S	DM	0.18	Bier & Müller 1969
P. apterus	1.32	F	23	FCM	WB	BeP	1.69	*Sadílek (unpub. data)
P. apterus	1.19	M	23	FCM	WB	BeP	1.69	*Sadílek (unpub. data)
LYGAEIDAE								
Oncopeltus *fasciatus*	4.65–6.15	-	16	BCA	WB	-	-	Lagowski et al. 1973

Supplementary table 2. Four genome size analysing studies of Schrader & Hughes-Schrader from the '50s presented results only in form of sample/standard ratio. Standard species was not specified, standard genome size was not given, therefore genome size cannot be expressed in picograms.

Method: FD = Feulgen densitometry

Cell type: TS = testes

2n = diploid chromosome number

Taxon	Sample/standard ratio	Sex	2n	Method	Cell type	Standard	Reference
Infraorder PENTATOMOMORPHA							
PENTATOMIDAE							
Acrosternum *hilaris*	3.81	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
A. marginatum	3.65	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
A. pennsylvanicum	4.02	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
A. scutellatum	3.61	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
Acrosternum sp.	3.13	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
Arvelius *albopunctatus*	2.19	M	14	FD	TS	mantid	Schrader & Hughes-Schrader 1956
Banasa *bidens*	1.53	M	26	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. calva	1.46	M	26	FD	TS	frog	Schrader & Hughes-Schrader 1958

Taxon	Sample/standard ratio	Sex	2n	Method	Cell type	Standard	Reference
B. dimidiata	1.10	M	16	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. euchlora	1.53	M	16	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. lenticularis	1.07	M	16	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. minor	1.56	M	26	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. panamensis	1.43	M	14	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. panamensis	1.43	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
B. rutifrons	1.44	M	26	FD	TS	frog	Schrader & Hughes-Schrader 1958
B. zeteki	1.31	M	26	FD	TS	frog	Schrader & Hughes-Schrader 1958
***Coenus** delius*	2.52	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
***Euschistus** crassus*	2.32	M	12	FD	TS	mantid	Hughes-Schrader & Schrader 1956
E. obscurus	2.58	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
***Loxa** flavicollis*	2.27	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
***Mormidia** lugens*	2.24	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
***Murgantia** histrionica*	1.82	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
***Nezara** viridula*	2.39	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
***Pallaea** stictica*	2.24	M	14	FD	TS	frog	Hughes-Schrader & Schrader 1957
***Peribalus** imbolarius*	1.80	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956
***Thyanta** antiguensis*	0.89	M	14	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. calceata	0.93	M	27	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. calcea x *T. pallidovirens*	0.84	M	22	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. custator	0.88	M	16	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. custator x *T. pallidovirens*	0.92	M	16	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. pallidovirens	0.98	M	16	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. perditor	1.10	M	14	FD	TS	mantid	Schrader & Hughes-Schrader 1956
T. pseudocasta	0.94	M	14	FD	TS	mantid	Schrader & Hughes-Schrader 1956
***Trichopepla** semivittata*	1.69	M	14	FD	TS	mantid	Hughes-Schrader & Schrader 1956

Paper I.

Extensive fragmentation of the X chromosome in the bed bug *Cimex lectularius* Linnaeus, 1758 (Heteroptera, Cimicidae): a survey across Europe

DAVID SADÍLEK, FRANTIŠEK ŠŤÁHLAVSKÝ, JITKA VILÍMOVÁ & JAN ZIMA

(2013)

Comparative Cytogenetics **7**(4): 253–269.

IF_{2013} = 1.101

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Author Contributions

DS and JV conceived and designed the research.
DS coordinated the material collection.
DS conducted the experiments.
DS and FŠ analysed the data.
JZ and DS wrote the manuscript.

Paper II.

Molecular cytogenetics of *Androctonus* scorpions: an oasis of calm in the turbulent karyotype evolution of the diverse family Buthidae

DAVID SADÍLEK, PETR NGUYEN, HALIL KOÇ, FRANTIŠEK KOVAŘÍK, ERSEN A. YAĞMUR & FRANTIŠEK ŠŤÁHLAVSKÝ

(2015)

Biological Journal of the Linnean Society **115**(1): 69–76.

IF_{2015} = 2.210

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Author Contributions

FŠ and DS conceived and designed the research.
HK, FK and EAY collected the material.
DS conducted the experiments.
DS and FŠ analysed the data.
DS, PN and FŠ wrote the manuscript.

Paper III.

Comparison of different cytogenetic methods and suitability for the study of chromosomes in *Cimex lectularius* (Heteroptera, Cimicidae)

DAVID SADÍLEK, ROBERT B. ANGUS, FRANTIŠEK ŠŤÁHLAVSKÝ & JITKA VILÍMOVÁ

(2016)

Comparative Cytogenetics **10**(4): 731–752.

IF_{2016} = 1.485

Doc. RNDr. Jitka Vilímová, CSc.

Author Contributions

DS, RBA and FŠ conceived and designed the research.
DS conducted the experiments.
DS analysed the data.
DS and JV wrote the manuscript.

Paper IV.

Investigation of the midgut structure and ultrastructure in *Cimex lectularius* and *Cimex pipistrelli* (Hemiptera: Cimicidae)

MAGDALENA M. ROST-ROSZKOWSKA, JITKA VILÍMOVÁ, LIDIA SONAKOWSKA, KAROLINA KAMIŃSKA, FLORENTYNA KASZUBA, ANGELIKA MARCHEWKA & DAVID SADÍLEK

(2017)

Neotropical Entomology **46**(1): 45–57.

IF_{2017} = 0.931

Doc. RNDr. Jitka Vilímová, CSc.

Author Contributions

MMR-R conceived and designed the research.
DS collected the material.
MMR-R, LS, KK, FK and AM conducted the experiments.
MMR-R, LS and FK analysed the data.
MMR-R and JV wrote the manuscript.

Paper V.

Nuclear genome size in contrast to sex chromosome number variability in the human bud bug, *Cimex lectularius* (Heteroptera: Cimicidae)

DAVID SADÍLEK, TOMÁŠ URFUS, JITKA VILÍMOVÁ, JIŘÍ HADRAVA & JAN SUDA

(2019)

Cytometry Part A **95A**: 746–756.

IF$_{2019}$ = 3.465

Doc. RNDr. Jitka Vilímová, CSc.

Author Contributions

DS, JS and TU conceived and designed the research.
DS coordinated the material collection.
DS conducted the experiments.
DS and JH analysed the data.
DS and JV wrote the manuscript.

Paper VI.

Genome size and sex chromosome variability of bed bugs feeding on animal hosts compared to *Cimex lectularius* parasitizing human (Heteroptera: Cimicidae)

DAVID SADÍLEK, TOMÁŠ URFUS & JITKA VILÍMOVÁ

(2019)

Cytometry Part A **95A**: 1158–1166.

IF_{2019} = 3.465

Doc. RNDr. Jitka Vilímová, CSc.

Author Contributions

DS and TU conceived and designed the research.
DS coordinated the material collection.
DS conducted the experiments.
DS analysed the data.
DS and JV wrote the manuscript.

Paper VII.

Peaceful revolution in genome size: polyploidy in the Nabidae (Heteroptera), autosomes and nuclear DNA content doubling

DAVID SADÍLEK, JITKA VILÍMOVÁ & TOMÁŠ URFUS

(Accepted manuscript)

Zoological Journal of the Linnean Society

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Doc. RNDr. Jitka Vilímová, CSc.

Author Contributions

DS and TU conceived and designed the research.
DS and JV collected the material.
DS conducted the experiments.
DS analysed the data.
DS and JV wrote the manuscript.