

Review of the dissertation of Mgr. David SADÍLEK

within the study programme *Zoology* at the Faculty of Science,
Charles University, Prague

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The author of the PhD thesis, provided me for review, David Sadílek was born in 4th November 1987 in Prague. He defended his Bachelor's (2010) and Master's (2012) degrees in Charles University, Prague. Since the Bachelor's period, the main subject of his interest and investigations is the blood-feeding ectoparasite bed bug *Cimex lectularius* (Insecta, Heteroptera, Cimicidae). This is the main subject in his PhD thesis too.

I know David Sadílek since the summer of 2012 when he took part in the 6th EHC in Blagoevgrad (Bulgaria) as a Master's student of doc. RNDr. Jitka Vilimová. At that time he already had results in cytogenetics of bed bug *Cimex lectularius* (Heteroptera, Cimicomorpha, Cimicidae) and especially the sex chromosomes polymorphism. Soon after that he defended his Master thesis and continued his investigations in different aspects. Within the next almost 10 years I followed with interest his publications, and his development. I was a reviewer and subject editor in some of his cytogenetic manuscripts.

In this period, David Sadílek wield a wide range of methods – he started with routine methods for chromosome slide preparations and their staining, and actively looks for new approaches (tissues, techniques, age, localities, relative groups) that can help him to answer the open questions, incl. modern molecular techniques such as FISH, PCR, FCM. In the his articles and the thesis one can see that he is familiar with the design of the experiment, he is able to make analysis and to interpret the results, as well to create ideas how to improve the protocols in the next experiments. David has already a rich history research work in grant projects, supported by the Charles University Grant Agency, as well some pedagogical experience with teaching of entomology at the Charles University.

The thesis, based on 7 published articles and a Supplement table with the genome sizes of all studied Heteroptera, is divided in 6 chapters – Introduction, Research methods, Cytogenetical and cytometric results, Conclusions, References.

The chapter Introduction provides literature data on the origin and distribution of the parasitism among Heteroptera, based on the cladograms of Weirauch et al. (2019). The author's attention is focused on both families, including ectoparasites – Reduviidae (in subfamily Triatominae) and Cimicidae (in genus *Cimex*) of the infraorder Cimicomorpha (Heteroptera), placing primary emphasis upon the parasitism evolution in the bed bugs, the connection between the bed bugs and their hosts – bats and humans (with an discussion of the hypotheses whether the ancestral cimicids were host generalists or specialists). Sadílek

underlines the economic significance of the damages caused by bed bug population in the human dwellings as one of the reasons the cimicids to be studied for years in different aspects. Some attention is put on the specific cytogenetic features of *Cimex lectularius*, such as their holokinetic chromosomes and the postreductional (inverted) meiotic division of the sex chromosomes (as in all Heteroptera). At the end of the chapter the insect genome size analysis by flow cytometry method (FCM) is discussed as another tool to understand the pattern of the chromosome changes' evolution and especially the origin of the X chromosome fragments in *Cimex lectularius*.

Second chapter, dedicated to the research methods which Sadílek wield during the period of his education and applied in his study, is divided in several parts – the techniques of chromosome slides preparation (a comparison between hotplate spreading technique and squash technique is done), the selection of the most suitable tissues for chromosome study (mainly the testes, sometimes ovaries, but not the midgut and eggs) and what is the best division phase for study of the chromosomes in *C. lectularius* with the aim to count the sex chromosomes in the pseudo-multivalent of the sex chromosomes. Because of the inverted sex chromosome meiotic division, at metaphase II the sex chromosomes form a pseudo-multivalent, what makes this phase a useful tool to prove that the chromosome polymorphism in *Cimex* concerns exactly the sex chromosomes (the X).

At this chapter, a special attention is paid on the role of FISH and FCM as approaches to analyse the chromosome slides and to find out a solution of different problems.

At the third chapter “Cytogenetical and cytometric results” the original results of the author, already published in seven his articles included in the thesis, are presented. It is divided in 5 parts – the distribution of the *C. lectularius* cytotypes today and in the past; chromosome variability in other cimicids; genome size of cimicid cytotypes; comparative research of the family Nabidae and comparative research of the *Androctonus* scorpions.

The analysis of the 12 found *C. lectularius* cytotypes distribution today and in the past is based on the data from the Master degree's thesis of David Sadílek (2012) and Sadílek (2013) for about 200 specimens from over 60 European localities studied and the data of the former authors – Darlington (1939) and Slack (1939). Sadílek (with co-authors) concludes that the *C. lectularius* cytotypes distribution is random and does not show any consistent geographic or phylogenetic pattern. In contrast to the data of Darlington and Slack published in 1939, David claims the main cytotypes distributed today are the basic one ($26+X_1X_2Y$) with 44% and two next – $26+X_1X_2X_3Y$ (20%) and $26+X_{1-4}Y$ (11%). The analysis of the chromosome number variability (the author calls it “chromosome variability”, but he is dealing in fact with the chromosome number variability) among the cimicids displays that it is available only in the species with X_1X_2Y sex chromosomes.

Sadílek considers the joint application of cytogenetical approach and FCM with plant standards and DAPI and PI staining as a very effective tool for studying the chromosome number variability. The results enable him to suggest that different chromosome arrangements

(not only fragmentations) take place in the chromosome evolution of the genus, and that the various cytotypes have different genome size.

A comparative cytogenetic research in the closely related to the cimicids family Nabidae is presented. It includes new data on the karyotype of earlier unstudied nabide species; the analysis of the distribution of the 18S rDNA in the karyotype of the nabid species and the genome size of species with 16 and 26/32 autosomes, respectively. The author considers the 18S rDNA distribution as a marker for similarity between Cimicidae and Nabidae – some of Nabidae species display signals on the both sex chromosomes (like *Cimex lectularius*), but there are other species in family Nabidae which show different position of the 18S rDNA in the karyotype.

Based on the genome size of species with 16 and 32 autosomes the author (with co-authors) claims that it is an argument supporting the autosomal polyploidy as the most probable pattern of the karyotype evolution in Nabidae. Working more than 10 years with this family I am not sure that this is the final solution of this long lasting discussion, but the future will provide maybe some more compelling evidences what hypothesis is true.

I have a question about the terms “population” and locality used in the text of this chapter – what the author think is correct term for his case – does he mean populations or it is simply not precise term?

In eight paragraphs of the chapter Conclusions the main results and author's suggestions are summarized. The majority of so called conclusions are in fact the summarized results of the study and not exactly conclusions – the information received on the cytotypes of *C. lectularius*, recorded within recent European strain from human host; the intraspecific variability in the number of the X chromosomes registered in *C. lectularius* from bats and *C. pipistrelli*; and the genome size for six cimicid species/strains; the chromosome formula of two earlier unstudied *Nabis* species (*N. biformis* and *N. maoricus*) and *Prosetemm aeneicolle*, the species specific pattern of 18S rDNA signals revealed in 12 Nabidae species, and the genome size of 8 species determined.

The conclusions are only the postulates that the fragmentations should be considered as the most common rearrangement in *C. lectularius* from bats and *C. pipistrelli* (not *pistrelli*), while in *C. lectularius* from human the most probable chromosome rearrangements are duplications and deletions; the suggestion that *Himacerus* karyotype is originated through autosomal polyploidy from the karyotype with 16 autosomes; as well as the evaluation of the methods for chromosome slide preparation and genome size determination by FCM.

In the chapter References there are 124 articles and books cited, incl. the seven articles of Sadílek and co-authors. The works cited represent the majority of the publications concerning the topic of the study and they are well interpreted in the thesis.

As a Supplement to the thesis a table with all data of the genome sizes available for representatives of Heteroptera is presented. Actually, as correctly mentioned by the author,

this is the template list of Gregory (2020) completed by the data of Sadílek's study and the missed data from eight other additional original articles.

THE ARTICLES ANALYSIS

P1

SADÍLEK D, ŠTÁHLAVSKÝ F, VILÍMOVÁ J & ZIMA J 2013: Extensive fragmentation of the X chromosome in the bed bug *Cimex lectularius* Linnaeus, 1758 (Heteroptera: Cimicidae): a survey across Europe. *Comparative Cytogenetics* 7(4): 253–269. (IF2013 = 1.101)

The manuscript of this article when submitted in the journal *Comparative Cytogenetics* had been reviewed by me and I provided the authors my remarks and suggestions at that time. My suggestions had been accepted by authors, so recently, I have nothing to add about the article. The results are original and I have no doubts they are received by David as a leader author. The article provides new and useful information about the polymorphism of the sex chromosomes (actually the X) of the *Cimex lectularius* across Europe, incl. 116 specimens of *C. lectularius* from 61 localities within 10 European countries and in five specimens of *C. pipistrelli* from two localities in Slovakia.

This is the first/oldest from the presented articles and naturally the routine method for the cytogenetic studies is applied, but it is enough informative for the aim of the study. Authors present 12 cytotypes of the *Cimex lectularius* – they confirm considerable variation in the karyotype of the bed bug and even extend its range (incl. the highest known chromosome number in the male bedbug karyotype with 47 chromosomes – $2n=26+X_{1-20}Y$), but individuals with lower numbers clearly prevailed.

The authors discuss the different published hypotheses about the origin of the supernumerary elements in the bed bug complements, but suppose that the most plausible explanation of their origin remains fragmentation of the X chromosome. The observed post-reductional behaviour of the fragments in meiosis (the behaviour of the sex chromosomes in bugs) and comparisons with other related species of the genus *Cimex* confirm this suggestion. Potential mutagenic effects of the insecticides and other toxic substances applied for treatment is presented as one of the explanations for the increased rate of chromosomal rearrangements in bed bugs, but there other factors which can influence this process too.

P2

SADÍLEK D, NGUYEN P, KOÇ H, KOVAŘÍK F, YAĞMUR EA & ŠTÁHLAVSKÝ F 2015: Molecular cytogenetics of the *Androctonus* scorpions: Oasis of calm in turbulent

karyotype evolution of the diverse family Buthidae. *Biological Journal of the Linnean Society* **115**: 69–76. (IF2015 = 2.210)

This article with intriguing title is published in a renowned international journal with IF2015 = 2.210. It is a result of successful cooperation of the authors from different regions and provides information about the stable cytogenetic peculiarities of four geographically distant species of the genus *Androctonus* (Scorpiones: Buthidae). The study includes a combination of the routine and molecular cytogenetic methods for examination of representatives of this basal arachnid order. The illustrations are in very good quality; the results provide new original information about the patterns of chromosome evolution in the family Buthidae.

Except the holokinetic chromosomes I could not find very serious reason to include this good article in the thesis titled “Cytogenetic characteristic of *Cimex* bed bugs”

P3

SADÍLEK D, ANGUS R, ŠTÁHLAVSKÝ F & VILÍMOVÁ J 2016: Comparison of different cytogenetic methods and tissue suitability for the study of chromosomes in *Cimex lectularius* (Heteroptera: Cimicidae). *Comparative Cytogenetics* **10**(4): 731–752. (IF2016 = 1.485)

For this article I was the academic editor of the MS and also provided my suggestions how to be improved the MS few years ago.

The article treats of mainly methodical questions. Sadílek and co-authors make an analysis of the suitability of the different cytogenetic methods for studies of the bed bug' chromosomes – the techniques of chromosome slides preparation (they compare hotplate spreading technique and squash technique); as well the selection of the most suitable tissues for chromosome study (mainly the testes, sometimes ovaries, but not the midgut and eggs) and provide their recommendations:

- better method for chromosome slides preparation in *C. lectularius* is the hotplate spreading technique, but it is inconvenient for the fixed material of other true bugs – unfortunately, often we have no possibility to prepare slides from fresh/alive material.
- best for study of chromosomes in *C. lectularius* division phase is MII when one has to recognize the pseudo-multivalent of the sex chromosomes.
- special attention is taken to the role of FISH and FCM as approaches to analyse the chromosome slides and to localize the position of certain gene/section of the genome in the chromosome.

This work will be very helpful for the further researchers who have to choose the most appropriated for the aim of their study methods and the tissues.

P4

ROST-ROSZKOWSKA MM, VILIMOVA J, WŁODARCZYK A, SONAKOWSKA L, KAMIŃSKAK, KASZUBA F, MARCHEWKA A & SADÍLEK D 2017: Investigation of the midgut structure and ultrastructure in *Cimex lectularius* and *Cimex pipistrelli* (Hemiptera: Cimicidae). *Neotropical Entomology* **46**: 45–57. (IF2017 = 0.931)

This is an interesting study on the midgut ultrastructure of hematophagous species with long intervals of starvation between blood meals and to compare two species from different hosts.

The results confirm the suggestion of former cited authors that a conserved digestive pattern appears in Hemiptera that ingest various kinds of food (fungusfeeding, detritus-feeding, seed-feeding, predaceous, zoophytophagous, phytophagous and hematophagous) and that it is not connected with insects' diet but is correlated with phylogeny and authors; and that the structure of the digestive tract of insects is not attributed solely to diet but to the basic adaptation of an ancestor.

The authors make important conclusions about the function of different parts of midgut:

- (1) the anterior midgut fulfils the role of storing food and synthesizing and secreting enzymes;
- (2) the medium midgut is the main organ for the synthesis of enzymes, secretion, and the storage of the reserve material;
- (3) the anterior midgut and medium midgut take part in water absorption;
- (4) the posterior midgut is the organ in which spherites accumulate;
- (5) the morphology and ultrastructure of the digestive epithelium have a conserved pattern in hematophagous as well as in non-hematophagous Hemiptera.

P5

SADÍLEK D, URFUS T, HADRAVA J, VILÍMOVÁ J & SUDA J 2019: Nuclear Genome size in contrast to chromosome number variability in human bed bug, *Cimex lectularius* (Heteroptera: Cimicidae). *Cytometry Part A* **95A**: 746–756. (IF2019 = 3.465)

To shed light to the problem with the extraordinary chromosomes and the chromosome number variability in the karyotype of the bed bug David applies molecular techniques, and especially flow cytometry method with plant standards, and staining with DAPI and PI as a very effective tool. The results received in studying of the various *C. lectularius* cytotypes allow him to suggest that beside the traditionally considered fragmentation of the X chromosome, other X chromosome rearrangements could be indicated to be the source of the additional chromosomes. There is a significant correlation between genome size and the number of chromosomes, but in some specimens with additional chromosomes, nuclear genome size decreases or remains average.

This method is not often applied for insects and especially true bugs and the results received are innovative and analysis of the methodical tests during this study could be of great importance in further application of FCM in investigations of the true bugs genome size. Some details, for instance, the differences of the behaviour in FCM of different tissues and sexes, tests with differently preserved material (freshly killed, dry, frozen and fixed specimens), the standard chosen, IP or DAPI staining and so on could be really very useful in further experiments.

I have no big experience with application of FCM and I am afraid I cannot evaluate in detail the experiment design and the results themselves, but I have no doubts such data is useful for studying the chromosome evolution.

P6

SADÍLEK D, URFUS T & VILÍMOVÁ J 2019: Genome size and sex chromosome variability of bed bugs feeding on animal hosts compared to *Cimex lectularius* parasitizing human (Heteroptera: Cimicidae). *Cytometry Part A* **95A**: 1158–1166. (IF2019 = 3.465)

Based on the occurrence of lower nuclear DNA content in *C. lectularius* (B) and *C. pipistrelli*, the authors claim that the additional chromosome cannot be the B chromosomes as discussed in earlier in their studies – it has been expected that B chromosomes should always increase the DNA content in all specimens with additional chromosomes in particular species. The specimens with distinctly lower nuclear DNA content suggested occurrence of some deleterious rearrangement as in *C. lectularius* from human –the most probable chromosome rearrangements are duplications and deletions.

P7

SADÍLEK D, VILÍMOVÁ J & URFUS T: Peaceful revolution in genome size: Polyploidy in Nabidae (Heteroptera), autosomes and nuclear DNA content doubling. *Zoological journal of the Linnean Society* 2020, zlaa138, <https://doi.org/10.1093/zoolinnea/zlaa138> (Accepted manuscript). (IF2019 = 2.842)

This article presents a complex study of 12 Nabidae species. The karyotype of all 12 species is determined (for 2 *Nabis* species and *Prostemma aeneicolle* the karyotype is published for the first time, the rest karyotypes are confirmed), some polymorphism in the chromosome number is reported; the chromosome measurements; FISH for localization of the 18SrDNA in the chromosomes and genome size of eight of the species studied. An analysis of the different hypothesis for the chromosome evolution in the family is provided.

I have some comments and questions:

- 1) *The authors say that the material is collected in 2013-2017 and that the chromosome slides are prepared from freshly killed specimens. I want to ask does*

this mean that the slides for this study were made years ago and if so, how the slides were stored till their staining and analysis (especially for FISH)? What was the situation with the material from New Zealand – was the material sent alive to make slides from freshly killed specimens?

- 2) *On fig. 1M, N one female mitotic metaphase of Prostemma aeneicolle is presented. I am interested to know what was the order of the treatments for this cell/slide – first FISH or first Giemsa – please, provide some methodic details?*
- 3) *In the article there is no information provided when the material was collected (I mean the date) – such information could give information why the males had less divisions. My experience with Nabidae chromosomes during the years is that the young males have huge testes with 7 follicles and one can make a lot of slides with divisions from them.*
- 4) *One more comment – in the article “postpachitene” is used for the stage when achiasmatic chromosomes condense – in the literature for true bugs with chiasmatic male meiosis this stage/phase is usually called “condensation stage”.*

This research has been executed to a very high standard. The candidate has clearly worked hard to produce large and complex datasets but has also managed to analyse these through a variety of techniques to distil out the principal drivers of the systems. The thesis is well written and error free (except some small technical errors).

In conclusion I want to say that the thesis and the articles presented display a well targeted research project. The author wields a wide range of methods, which he critically applies and with competence interprets the results received. I strongly recommend the work and the PhD degree to the author David Sadílek.

Sincerely,

Prof. Snejana Grozeva, PhD