

Posudek habilitační práce RNDr. Evy Novákové Ph.D.

*Insect symbiosis: insights into ecology, phylogenetic diversity and evolutionary dynamics*

v oboru **parazitologie**, Přírodovědecká fakulta Jihočeské univerzity v Českých Budějovicích

Habilitační práce RNDr. Evy Novákové Ph.D. je zpracována v anglickém jazyce v méně běžném formátu komentování dosažených výsledků. Přiznávám, že mi tento způsob vyhovuje méně než standardní členění na úvod, materiál a metody, výsledky a diskuzi. Přestože celý habilitační spis má 249 stran a na první pohled se jeví jako rozsáhlý, nového textu ve smyslu komentovaných výsledků je pouhých sedm stran (po odstranění literárních odkazů, obrázků a při použití jednoduchého řádkování). Těchto sedm stran je navíc rozděleno do dvou kapitol, z nichž každá má svůj přehled citované literatury. Seznam literatury na straně 8 a další pak na straně 15 mne poněkud zaskočil a ke srozumitelnosti celého spisu takové strukturování nepřispělo. U habilitačního spisu bych si přece jen představoval trochu rozsáhlejší pozastavení se na doposud získanými výsledky.

Před posuzováním habilitačního spisu jsem si nejprve otevřel soubor „ThesisSummary“, zevrubně jsem si jej přečetl a teprve pak jsem postoupil plné verzi habilitace. Byl jsem však nepříjemně překvapen, že text představující habilitační práci je v souhrnu i ve vlastní habilitační práci naprosto identický. Souhrn tedy není souhrnem, ale habilitační práci bez příloh ve formě jednotlivých publikací.

Veškeré texty jsou v anglickém jazyce, což bude většina lidí hodnotit nadšeně pozitivně. Já váhám a to z následujícího důvodu. V takovém případě nedokážu odlišit, zda nesrozumitelnost některých vět je důsledek použití cizího jazyka anebo důsledek slabší orientace autorky a její neschopnosti přesně popsat a vystihnout myšlenky. Autorka má oblibu v dlouhých větách se složitou větnou strukturou. Volba některých slov vyústila v dvojmyslná sdělení a takové věty jsem musel číst i třikrát, než mi došlo, co autorka zamýšlí. Jako příklad uvádím slovo „predating“, které autorka použila ve smyslu predating, tedy myšleno časově, před nějakým časem. Já jsem však danou větu a slovo chápal ve smyslu predátor a predace. Na jiném místě se vyskytnul termín „radical degeneration“, který jsem chápal ve smyslu „odbourání (kyslíkových) radikálů“. Autorka tato slova použila pro vyjádření českého významu „radikální (rozsáhlá) degenerace“. Můžete argumentovat, že to je moje chyba, ale není. Odborný text musí být jednoduchý a nesmí umožňovat dvojitý výklad. Podobně mi nevyhovovalo spojení Outstanding Aphids, protože nevím, co na nich je outstanding. To je velmi subjektivní a ne zcela vhodné i přesto, že autorka takový výraz použila s dobrým úmyslem.

Naštěstí tohle jsou poslední výtky, které k habilitačnímu spisu mám. Odborné pojednání o vztahu různých hmyzích druhů s baktériemi bylo originální a i přes výhrady k jazyku se dobře četlo. Nakonec mi bohatě stačilo i těch sedm stran textu k tomu, abych pochopil, čím se autorka zabývá – a bavilo mne to. Dosažené výsledky mají vysokou úroveň bohatě

dosahující kritéria pro udělení titulu docent/docentka. Dr. Nováková má i přiměřenou publikační činnost, což dále usnadňuje mé rozhodování, protože každá z jejich publikací byla anonymně posouzena minimálně dvěma mezinárodními odborníky v daném oboru. Proto k odborné části nemám žádné kritické připomínky.


Na autorku mám tři dotazy.

1. Lze některé ze symbiotických baktérií kultivovat in vitro na tradičních živných agarrech a tekutých půdách?
2. Dokáží si vysvětlit, že pokles symbiotické *Wolbachia* u komára *Culex pipiens* může vést k jeho větší vnímavosti k West Nile virus. Jak se ale vysvětluje vyšší mikrobiální diverzita u *Trypanosoma cruzi* pozitivních ploštic *Triatoma protracta*?
3. Ve vaši práci Chrudimský et al. *Candidatus Sodalis melophagi* sp. nov.: phylogenetically independent comparative model to the tsetse fly symbiont *Sodalis glossinidius*. PLoS One. 2012;7(7):e40354 zmiňujete přítomnost type III secretion system u *Sodalis* sp. Struktura těchto systému je téměř identická s TSSS u *Salmonella enterica*. Existují nějaké hypotézy, jak se tento ostrov patogenity mohl dostat současně do *Sodalis* i *Salmonella*? Já jsem doposud považoval tento systém (myšleno geny tvořící tento ostrov patogenity jako je *hilA*, *invA* atd.) za naprosto *Salmonella* specifické. A neměl by se tento genomický ostrov přejmenovat na ostrov symbiózy?

Shmutí posudku:

Práce RNDr. Evy Novákové Ph.D. splňuje požadavky na udělení titulu docent/docentka a proto doporučuji pokračovat v habilitačním řízení habilitační přednáškou a obhajobou habilitační práce.

V Brně dne 2. září 2018

  
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**Review of the habilitation thesis "Insect symbiosis: insights into ecology, phylogenetic diversity and evolutionary dynamics" by Dr. Eva Nováková.**

Opponent: Alexei Kostygov

The thesis presented by Dr. Eva Nováková is devoted to the multifaceted study of bacterial endosymbionts of insects. At the first glance, this subject may seem to be rather microbiological and therefore far from the field of parasitology, which is the formal specialization of the author. Nevertheless, when taking into account that the hosts of the considered symbiotic bacteria are parasitic (in a broad sense) insects everything falls into place.

The significance of bacterial endosymbionts for the adaptation of their hosts to various ecological niches can hardly be overestimated. Endosymbiosis represent one of the fundamental phenomena, driving the evolution of life on Earth. The origin of eukaryotes and then algae would be impossible without endosymbioses with bacteria, which eventually turned into organelles (mitochondria and plastids, respectively). Numerous subsequent gains of endosymbiotic prokaryotes promoted diversification of life forms in various groups of eukaryotes. In case of established mutualistic relationships, endosymbionts complement the metabolic abilities of the host, that is to say provide it with essential nutrients, thereby allowing it to consume compositionally restricted food, such as, for example, plant sap. However, the influence of symbiotic bacteria is not only confined to this, especially given that not all of them are mutualists and not all of them are permanently present in the host. Thus, it is not surprising, that studies in this field are very important for the comprehension of the biology of a particular group of hosts. In the new century, this research was further boosted by the advent of massively parallel DNA sequencing technologies and concomitant progress in bioinformatics methods making analysis of bacterial genomes a routine.

Dr. Eva Nováková is one of those who succeeded in this significant field of study, as justified by her decent publications including 2 book chapters and 12 research articles in impact-factor journals (7 as the first author and 5 as corresponding author, partially overlapping). Her findings provide important insights into the origin and evolution of endosymbiotic bacteria of insects, their influence on hosts' biology and even phylogeny of the hosts themselves.

The thesis starts from an introduction representing an extended summary of the work. It is subdivided into preface and two chapters. The preface explains the importance of the subject and the career history of the author, wherefrom the subsequent subdivision of the work becomes clear. The two chapters differ in the host groups: the smaller first one is related to sap-feeding aphids, whereas the larger second one concerns mostly various blood-sucking insects such as lice, kissing bugs, mosquitoes as well as tsetse and louse flies. The Chapter 1 is based on three articles: one of them proposes ingenious solution to the issues of resolving high-level phylogeny of aphids by using the molecular data from the vertically transmitted obligate endosymbiotic bacteria. The second article scrutinizes evolution of carotenoid genes and demonstrates that they were gained by horizontal transfer from fungi. Although that is not directly related to the thesis subject, the inclusion of the paper is justified, since previously a hypothesis was proposed, that these genes could be acquired from bacterial endosymbionts. The third article is about successful gene silencing in aphids using RNA interference. Frankly speaking, I did not understand why it was included.

The Chapter 2 has four sections, which comprise the remaining 9 articles and 2 book chapters and therefore represents the main part of the thesis. It contains characterization of particular species of endosymbionts and their comparison with those from other hosts using genomic and functional traits, accompanied by discussion of the factors leading to convergence or discordance. Some publications also consider diversity and entangled evolution of symbiotic systems in insects with multiple gains, losses, replacements and horizontal transitions of endosymbionts belonging to various bacterial genera. Of special interest is the book chapter concerning the issues in studying host-endosymbiont coevolution and revealing events violating it. This detailed review can be useful even outside the studied subject. In addition, two articles consider seasonal and ontogenetic microbiome dynamics in triatomines and mosquitoes as well as the influence of this factor on vector competence. With no doubt these results are of practical significance, since

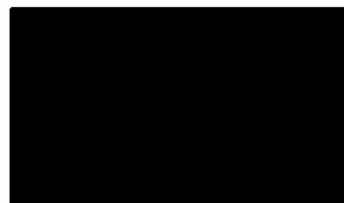
the transmitted infectious agents in question are human pathogens West Nile Virus and *Trypanosoma cruzi*.

In sum, I thoroughly enjoyed reading this thesis and obtained a lot of interesting new information. In my opinion, the necessary requirements of the University of South Bohemia the degree of docent (associated professor) have been fulfilled. Therefore, I recommend awarding this degree to Dr. Eva Nováková. For me it was an honour and pleasure to be a reviewer of this thesis.

Questions:

1. Could you elaborate the issue with the nomenclature of *Buchnera*? Why only one species *B. aphidicola* is recognized within this genus, given that its representatives for a long time coevolved with aphids? I would expect each aphid to have its own species of endosymbiont.
2. Why the article about RNA interference was included into the thesis?
3. The following sentence is confusing, could you explain, what does it mean?  
"Parasitism is simply intertwined with symbiosis on different levels and, by a more general view of a common interaction between unrelated organisms, even understood as a form of symbiosis itself."  
Parasitism indeed represents one of the forms of symbiosis and this is a commonplace.
4. Are there any other known examples of the microbiota influence on the vector competence / susceptibility to pathogens of their hosts?

Ostrava, 1 October, 2018



Alexei Kostygov, Ph.D.

Opponent's review of Habilitation thesis of: **Dr. Eva Nováková, PhD.,**  
**Faculty of Science, University of South Bohemia, České**  
**Budějovice, Czech Republic**

***Insect symbiosis: insights into ecology, phylogenetic  
diversity and evolutionary dynamics***

in the field of **Parasitology**, Faculty of Science, University of South Bohemia in České  
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**Review**

This thesis constitutes a series of excellent papers, both research and some overviews, published in international high-impact refereed journals. In effect, the quality of the published work speaks for itself. I have nothing but praise for the careful science planned and performed, nor its more specific conclusions, which seem both sensible and perfectly reasonable to me

However, rather than find fault with the thesis itself and the largely molecular research work therein, I think it not amiss to consider how some of the findings relate to other, perhaps broader aspects of biology, and in so doing, ask the candidate, Dr. Nováková, for her views on these matters.

With regard to the mutualistic/symbiotic-parasitic bacteria species described and discussed, some association between bacteria and insect hosts appear ancient, as revealed in terms of congruent phylogenetic trees, e.g. aphids and their bacterial symbionts (co-cladogenesis). Others though, are much more recent, or so it appears from her and colleagues' studies, e.g. *Arsenophonus* spp., with transmission possibly via horizontal gene transmission (HGT) as well as maternally and vertically via eggs to the next generation, and which may distort the sex ratio of the host. The research shows that the insect hosts are rather like humankind as related by the late 16<sup>th</sup>/early 17<sup>th</sup> century English metaphysical poet John Donne (1572-1631) "*No man is an island entire of itself.*" Similarly, the fundamental biology of the organism concerned – blood sucking or phloem sucking, hence both parasitic is intricately and intimately associated with symbiotic bacteria, free living or residing in more evolved structures in the gut, bacteriocytes.

In the very host-dependent bacteria like *Buchnera* and *Wigglesworthia*, the genome is greatly reduced and the bacteria relies on the host or host's meal (phloem in the case of herbivores, blood in hematophagous insects) to supply necessary nutrients, and often as not, *vice versa*. This all points to a finer and finer-grained co-evolution between bacteria and insect host and perhaps even the apparently 'generalist' species of bacteria like *Arsenophonus* and *Sodalis* are on a one-way ticket to unique specialisms whereby they,

like *Buchnera* in the case of aphids, become totally dependent on the host for metabolic support as well as a residence (Henry *et al.*, 2013; Alkhedir *et al.*, 2015; Sugio *et al.*, 2015; Frantz *et al.*, 2016).

In the case of Tsetse flies (*Glossina* spp.), the mutualistic bacterium *Wigglesworthia glossinidia* provide nutrients (the B vitamin thiamine) for the development of the viviparous larvae fed via a specialised ‘milk gland’. Some of this insect-bacterial association is so ancient and fundamental that, as with aphids, the insect host is totally dependent upon their presence and presumably cannot survive if the bacteria is eliminated with antibiotics such as oxytetracycline.

*Is this true of these and indeed for all such insect symbionts, do you know?*

The degree of association puts a new perspective on the question of generalism *versus* specialism. If the symbiotic bacteria are so very essential for the welfare and survival of the host insect, and indeed whether intimately involved in fundamental biochemical-physiological processes related to the insect host’s diet, be it phloem or blood, can we consider such hosts to be ‘generalist’ in any sense of the word? My own feeling is ‘No, we can’t’. Dr Nováková shows that certain bacteria such as *Arsenophonus* and *Sodalis* appear to be more free-living (and indeed some species can be cultured *in vitro*; e.g Mathews *et al.*, 2005) and hence able to transfer between more diverse insect hosts than the more specific and genetically-reduced bacteria like *Buchnera* and *Wigglesworthia*. The gene complexes they have are not always involved in the production of a flagellum necessary for penetration into host cells. Some are more free-living in the insect body than others or confined to insect cells. Either way, the bacteria become integrated with the host to a greater or lesser extent and one has to conclude that their presence, if not actually parasitic, is of benefit to the insect host [and interestingly in this context, Dr. Nováková cites her published work showing that the presence of intracellular bacteria *Wolbachia* actually reduces the infection ability of certain mosquito spp. to carry and hence transmit Western Nile Virus (WNV), related to ambient temperature; *cf.* Fig 8 in Nováková *et al.*, 2017; *loc. cit.* ].

Further in this light, Dr. Nováková’s work seems to be very pertinent to the whole topic of insect cryptic species complexes and biotypes (e.g. Eastop, 1973; Loxdale & Harvey, 2016; Loxdale *et al.*, 2016). One might reasonably ask whether these biological entities are often a manifestation of the unique development of co-evolutionary associations between bacteria and insect host? In other words, is the fact that some aphids, for example the peach-potato aphid, *Myzus persicae* (Sulzer), an *apparent* ‘generalist’ attacking plants within 40 families, *really* an array of cryptic host-adapted biotypes or even higher evolves levels of ecological-evolutionary divergence, with unique symbiotic bacteria? (Loxdale *et al.*, 2011). The coupled aphid-bacterial correlated phylogenies points in this direction. Otherwise, one has to maintain the (as yet unsubstantiated) belief that the same aphid species *sensu stricto* can truly deal with and utilise an array of phloems with different amino acid content (both qualitatively and quantitatively), as well as deal with many diverse secondary plant anti-feedant chemicals of diverse chemical structure, i.e. with very different structures and hence chemical bonds (Loxdale *et al.*,

2011). Thus do the symbionts actually provision the necessary metabolites, e.g. essential amino acids, as well as directly detoxify and metabolise the antifeedants? Or rather, is this essentially a function of the aphid's own (one could argue) 'specialist' biochemical-enzymic abilities? (Loxdale & Balog, 2018)

*Can the candidate possibly throw any light on these possibilities?*

Which brings me inevitably onto the topic of the lost genes of some bacterial symbionts, as with mitochondrial DNA (mtDNA), i.e. Numts (nuclear mitochondrial DNA segment).

*Are these genes integrated at all with the host insect genome or not, the latter appearing to be the case with the semi-free-living bacteria?*

Will this bacterial genome or parts of it be eventually subsumed into the host genome and become one with its symbiont, a bit like mtDNA and many regions of the genome of complex eukaryotes, where genomes are seemingly largely comprised of ex-viral and ex-bacterial genetic sequences, so-called 'junk DNA'. Is the integration of insect host and insect bacteria a means to an end, or just by chance, the end of the free-living bacteria *per se*. It is a somewhat disturbing thought that the mutualistic-parasitic bacterial genome ultimately becomes the host, whether it likes it or not!

*Would Dr. Nováková like to comment on this point?*

Moving on and to address another major topic of the thesis, the carotenoid pigments of aphids, the data presented shows that different aphid species have very different and indeed distinct profile/s (*cf.* Fig. 4 in Nováková & Moran, 2011; *loc. cit.*). What does this mean exactly, any more than the colour patterns of the wings of butterflies and moths? In the case of the latter, they have an adaptive purpose – sexual dimorphism, crypsis, defence, heat regulation, etc. What of aphid colouring? (Dransfield & Brightwell, 2015; Tsuchida, 2018).

*Can Dr. Nováková throw any light on this issue?*

For example, many aphid species have polymorphic colour forms. The grain aphid, *Sitobion avenae* is mainly brown and green, but actually has a spectrum of colours – ranging from apple green to pink, red, chestnut brown to almost black! (Jenkins, 1991; Jenkins *et al.*, 1999). Why such variation? How is this regulated if the genes responsible are only gained, via HGT, from a fungus? There must surely be mechanisms regulating the genetic architecture along the genome and the expression of the said genes. There is now information on this regulation and gene expression (Zhang *et al.*, 2018). What are the colours for exactly – crypsis, ant attendance, solar radiation protection? (Jenkins, 1991; Jenkins *et al.*, 1999; Watanabe *et al.*, 2016). Not only these aspects, but there are sexual differences in colour (male aphids are often pink and oviparae green) and the colours can be changed in relation to temperature and day length (Jenkins, 1991; Jenkins *et al.*, 1999; Alkhedir *et al.*, 2010). In pea aphids (*Acyrtosiphon pisum*) a single bi-allelic gene seems to be involved (Caillaud & Losey, 2009), but clearly the system is



more complicated, certainly in other species, or so it appears (*cf.* also Mandrioli *et al.*, 2016). What about, for example, when green mothers give birth to green offspring? (Jenkins, 1991).

*Has Dr. Nováková any views on this? Do you think an epigenetic process may be also at work here?*

I can happily believe that some of the carotenoids of aphids derive, via HGT, from a fungus, but why can't the yellow/green carotenoids, commonly found in aphids, derive from their plant host/s? (e.g. Wang *et al.*, 2014). They seem to be in other closely related insects such as whiteflies (Sloan & Moran, 2012).

*Does Dr. Nováková wish to say anything on this?*

Returning to the bacteria maintained in the bodies of the insect parasites of warm-blooded vertebrate hosts, it may sometimes be quite difficult to transfer as a Hippoboscid fly/keed, e.g. *Melophagus ovinus* from one host to another – leading to repeated severe population bottlenecks, which must undoubtedly cause purging of the genome by genetic drift (e.g. Glémin, 2003; Althoff *et al.*, 2014).

*Do you think that this is/may be a problem in such insect parasite populations?*

Is this perhaps the reason why the bacterial genome is often reduced significantly in such mutualistic/parasitic associations? Maybe it is not so much of a problem in maternally inherited, vertical transmission, but may be more so in HGT (?).

Lastly, what comes first? Parasitism or mutualism? And indeed, does Dr. Nováková believe that the acquisition of these various bacteria, by whatever means, vertical or horizontal, governs the future direction of the host insect concerned down various specific (i.e. specialist) ecological avenues or, is the insect host heading in that particular direction anyway and the bacterium/bacteria just assist the process along by providing nutrients in the case of symbiotic/mutualistic species? Or is this too simplistic a view? It is a bit like: Does a new species always create a new ecological niche (sometimes by replacing/outcompeting the original incumbent, as the candidate suggests in certain bacterial/insect host cases she cites, or does it (the bacterium) sometimes fill a vacant niche? (i.e., like Albatrosses filling the niche once occupied, we assume, by sea-going pterodactyls, and dolphins the niche once occupied by ichthyosaurs).

*Does your data give any clues to the ecological-evolutionary processes involved?*

Once the parasitic/mutualistic way of life is adopted, is the co-evolved bacteria-insect host pair on a one-way track to extinction...on the basis that severe specialism inevitably leads to extinction?

*Is this true, do you believe?*

As Charles Darwin suggested with the fossil record, it is incomplete because not all individuals of a particular mutated species are necessarily fossilized and indeed eventually found.

Is the sometimes apparent lack of synchrony/congruence between bacterial and insect host genomes a kind of similar process, i.e. not all the possible associations have been seen and documented and hence our knowledge is still incomplete and perhaps may always be so?

*What are the big future areas of your research that you wish to explore and why?*

## Conclusions

I congratulate the candidate Dr. Nováková on her excellent research and clear, well-written and indeed fascinating papers, collected together in this Habilitation thesis. I personally learnt a lot from reading the thesis and my only additional comment is that I hope that she considers further broadening the reach of her future findings to try and answer some of the major problems related to ecological adaptation and thereby evolution, more especially in terms of specialism vs. generalism (true, a current 'pet' topic of mine!).

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**Date:** 5<sup>th</sup> October, 2018

**Signed**



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