prof. Ing. Dušan Gömöry, DrSc., Technická univerzita vo Zvolene, Lesnícka fakulta, TG Masaryka 24, 96053 Zvolen

Review of the PhD. thesis of Ing. Josef Janoušek, Mendel University in Brno, entitled "Population biology of the pine needle pathogen *Lecanosticta acicola* (Thürm.) Syd. (Capnodiales, Ascomycota)"

The thesis is interesting and addresses a highly relevant topic – biology and invasion potential of an important fungal pathogen of a widespread and economically important tree genus.

There seems, however, to be some discrepancy between the original Czech title ("Rozšíření, hostitelské spektrum, ekologie, patologie a genetická struktura *Lecanosticta acicola* v ČR a zhodnocení současného stavu v Evropě"), as given in the nomination letter of the dean of the faculty, and the English title of the thesis. Whether 'distribution, host spectrum, ecology, pathology, genetics and the present state in Europe' all can be subsumed under 'population biology' is a question. This is not only a matter of the title. The thesis is composed of one published paper, one manuscript submitted to *Environmental Microbiology*, and a general introduction, summarizing the problem, objectives, methodology, results and their interpretation. Such concept is generally preferred in Europe and I myself prefer it as well, I do not like theses composed as 'monographs'. For selfish reasons, admittedly: in this case, the role of the reviewer is much easier, as a good portion of the thesis has already been subjected to peer review and usually does not need detailed inspection. However, the introductory part of such thesis should not be conceived as a scientific paper, but rather introduce a broader context of the thesis.

Considering this, the effort of the candidate to be brief and concise in the introductory part (which, in general, is a praiseworthy effort) is a bit exaggerated. A part of my criticism is associated with the fact that some parts of the introductory section were literally copied from the 2nd paper including typing errors (e.g., '*Samples were collected from the Americans...*' instead of '*Americas*' – p. 4). What is more important, there is much important information missing in the introductory section, especially for a person like me, who is not familiar with mycology. I am quite sure, that the candidate disposes of this knowledge, but did not include it into the thesis. The aspects of the study mentioned in the Czech title are not redundant. In fact, the knowledge of distribution, host spectrum, ecology, pathology is necessary to understand population biology of any organism.

From the formal point of view, the introductory part often refers to facts and results, which are presented in the attached papers only. Searching for them requires browsing there and back – it might have happened that I overlooked some of them although they are present somewhere in the text and that some of my questions and remarks are obsolete. Also inconsistent designation of populations (paper 2: p. 12 CE, MS, NA, SE, table S2 CA, CE, HA, HE) made reading difficult. What I clearly did not like was the typography – reading most tables written in minuscule fonts and figures with small symbols (e.g., pie graphs in fig. 2) was a hard nut for an old, weak-eyed man like me.

Back to the content – for example, nothing is written about the physiological mechanisms by which the fungus damages the needles – which life processes are affected, in which way and by which mechanisms? What is the ecology of the fungus? Does the fungus survive everywhere, where pines can be found? Is it hosted by all pines, or by a subset of pine species? What are the details of its life cycle? Etc. etc. – answers to these questions are in fact an important background for judging the

invasiveness of the fungus, and, consequently, judging how realistic are the outcomes of the ABC analyses. I suggest that these issues are <u>briefly</u> addressed during the presentation of the thesis – in that case it is not necessary to answer the above questions.

What I also miss in the thesis is addressing the last issue mentioned in the Czech title, namely the evaluation of the present state of fungal attack in Europe. The field, in which the thesis was submitted for defense, is not biology, but forest phytology as a part of agricultural and forestry sciences. Tackling practical issues is thus unavoidable. The impression which I got from the introduction is that the fungus is a problem in North America, partly China, but not so much in Europe. *Pinus sylvestris* is mentioned among host species, but only in North America, no samples from native stands in Europe are mentioned in Table 1. Generally, from what is written on p. 3, it seems that plantations of exotics rather than native stands are attacked – is it so? May the even-aged stand structure typical for plantations contribute to the spread of the fungus?

Another part where the excessive brevity is not very helpful, is the description of the materials used for study and methods. One of the tasks of the reviewer as well as the commission is judging to what extent the thesis has resulted from the work of the candidate, and this must be clear from the thesis itself. I would not open this issue unless I saw that in Table 1, there is a list of isolates including the names of the collectors, and the name of the candidate is listed only in 5 cases. This is, of course, nothing unusual and nothing wrong. However, the text does not show which steps following the sampling, i.e., steps in laboratory analyses and data treatment, were done by the candidate himself. Did the candidate receive isolates and made everything else starting from the DNA isolation by himself? If so, everything is OK. If not, the contribution of the candidate should be clarified.

Moreover, I would expect justification of the choice of methods. The use of Approximate Bayesian Computation is justified in the Introduction, but what about the other methods? Why Structure, and not, e.g., BAPS? Why EF, and not, e.g., ITS? Why F_{ST}, and not R_{ST}?

Specific comments:

Table 1: Scots pine is mentioned only in the case of two isolates from North America; among European samples, only *P. mugo*, *P. uliginosa* and exotics are listed. Does it mean that the fungus does not attack native stands of Scots pine in Europe?

Section 3.4 why just the EF region was chosen for sequencing?

Section 3.6: the symbols (e.g., K) and abbreviations (e.g., MLH) should be explained at the first use. Structure: Was the admixture or no-admixture model used in the Structure analysis? Why the delta-K method of Evanno et al. was not used as one of the criteria for determining the true (best supported) K value? PCA is mentioned – what data were analyzed by PCA and where are the outcomes presented? '*Genetic differentiation was calculated...*' – what was calculated? Genetic distances? F_{ST}? R_{ST}? If the text refers to Table 2 in the paper no. 2, it seems to be F_{ST}. Why? There is no unanimity about the proper mutation model of microsatellites, but for sure it is not infinite allele model. R_{ST} better reflects mutational process under SMM or TPM. Where are the results? The mentioned Table 2? Differentiation is potentially affected by linkage disequilibria and null alleles – were the datasets tested for both?

Section 3.8: I do not properly understand the description of some scenarios in Table 4/paper 2 and fig. S3. What means 'introduction A→E (or E→A) of each lineage separately'? The lineages must have originated from somewhere, there must have always been an ancestral lineage from which they split. Or do the scenarios 1 to 3 suggest polyphyletic origin of the fungus? I also do not understand the

colors in the graphical presentation of the scenarios (fig. S3). For example, scenario 1 evokes in me an impression that the MS population is the ancestral one and has remained unchanged until the present, the NA lineage diverged from it first, the SE lineage later, and finally CE split from the NA. This is something different than the scenario 1 as described in Table 4.

Section 4.5: Did the amplification of Central American samples fail with all eleven markers? Was the amplification of Colombian samples successful? Because if so, I suspect a technical failure rather than a novel taxon to be the reason.

Section 4.6: Fig. 4 – some of the structures appearing at $K \geq 4$ are clearly artefacts. Would not the analysis at $K=3$ and then looking for substructure by separate analyses of the blue and yellow groups be a more reasonable strategy?

Section 4.7: Table 4 – what measure of allelic richness was used? Commonly, allelic richness is expressed as the number of alleles after adjustment for sample size by rarefaction. In this case, naturally, A_R cannot be less than 1 (there cannot be less than 1 allele at a locus). Which diversity index was used to calculate genotypic diversity?

Table 4 indicates extensive clonality in this fungus. In the light of this, what is a 'generation' in ABC scenarios? Does a generation last between two mating events or between two propagation events? And what is the (average) generation turnover time in this fungus?

In spite of all this criticism, my answers to question formulated in the nomination letter are positive:

a) The thesis is based on appropriate methodological approach. The material used for the study is broad and I only can congratulate the candidate for being able to gather samples covering sufficiently the whole distribution range. The laboratory analyses covered a broad spectrum of methods (microsatellites, including nSSRs development, mating-type markers, sequencing) and the data treatment shows that the candidate is familiar with contemporary statistical methods. I suppose that the modification of the title was approved by the supervisor and faculty; the objectives, as formulated in section 2, were fulfilled.

b) The outcomes of the thesis represent a nice piece of good science. The candidate contributed both to the development of analytical tools and to elucidation of the evolutionary history of an important pathogen of forest trees.

c) The thesis has a direct relevance for forest phytopathology both from the theoretical and practical point of view. The knowledge of the genetic structure within a pathogen species may be important for fighting it, as genetically similar populations are expected to show similar response to any treatment.

d) The thesis has a few weak points, but as a whole, it clearly fulfills the requirements of a doctoral dissertation. I recommend accepting it and in case of a successful defense, awarding the candidate the academic title *Philosophiae Doctor*.

Zvolen, 11 March 2015

(Dušan Gömöry)