

## Posudek na magisterskou diplomovou práci Radky Jungové

The prevalence survey of oak powdery mildew *Erysiphe alphitoides* in Europe using molecular markers

Radka Jungová se ve své práci zabývá evropskými populacemi invazního padlí dubového (*Erysiphe alphitoides*), význačného patogenu dubu letního. Jako svůj cíl si vytyčila ověřit druhový status 30 vzorků z Evropy pomocí segmentu ITS, posléze navrhnout primery pro mitochondriální DNA sledovaného padlí a nakonec zhodnotit použitelnost vybraného segmentu mitochondriální DNA.

Na první pohled úsporná práce je chvályhodně sepsaná angličtinou, které se ovšem dle mého laického názoru nevyhnuly občasně gramatické kiksý. V diplomové práci by bylo při komentování vlastní práce diplomatičtější používat spíše jednotné číslo, než množné. Ucházející úprava textu je, alespoň v moji kopii práce, poněkud v kontrastu s některými nekvalitními obrázky. Literární přehled dostatečným způsobem uvádí čtenáře do problematiky, nelze však přehlédnout jeho zhruba pětinasobný rozsah oproti diskuzi, naznačující nevyváženost práce. Za poměrně závažný problém považuji značně nestandardní způsob citování literatury, kdy autorka hojně cituje práce se dvěma více autory pouze uvedením prvního autora a roku publikace. Přehled literatury je nejednotný a v citacích se občas vyskytují překlepy. Četné překlepy jsou i v použitých odborných termínech a latinských názvech, což nevyhnutelně snižuje celkový dojem. Pokud jde o cíle práce, autorka je sice poměrně jasně vymezuje, avšak v závěru práce na ně odpovídá spíše rozpačitě. Na jasně zmíněný cíl by měla být jasná odpověď.

Autorka by měla věnovat výrazně větší pozornost popisu metodiky. Čtenář se nedozví takřka nic o sběru vzorků, natož o charakteristice sledovaných lokalit, které předvádí pouze drobná mapa s přehnaně velkými značkami. Není jasné, zda autorka sbírala vzorky v různých zemích Evropy sama, v jakém množství, ani kde přesně se nacházely stromy na lokalitách určených jen jako název obce a někdy ani to ne. Velmi stručná diskuze obsahuje pasáže vhodné spíše do metodiky. Autorka jen velmi stroze odkazuje na klíčové fylogenetické stromy (obr. 6, 7 a 8) s nedostatečnými popisky. Čtenář například jen obtížně odhadne, čím se liší stromy na obr. 6 a 7. Diskuze je celkově nekonzistentní, občas není jasné, o čem autorka zrovna mluví nebo na který odkazuje obrázek.

K práci mám následující dotazy:

Co znamená odkaz SEM for example na s. 4?

Proč si autorka vybrala právě ty dva dotyčné molekulární markery? (s. 9)

Co mají společného, kromě původu z tropů, rody *Anacardium*, *Bixa*, *Citrus*, *Mangifera*, *Acacia*, zmíněné na s. 4?

Podle čeho si autorka vybrala použité outgroups? (s. 14)

Jak velké datasey byly použity pro fylogenetické analýzy? (s. 14)

Proč autorka použila 1000 replikací bootstrapu pro MP a 100 pro ML? (s. 15)

Proč není na obr. 6 uvedena ani jedna hodnota bootstrapu? Pokud ani jedna větev není dobře podložena, pak autorka neměla tvrdit, že je z takového obrázku něco „evidentní“. (s. 18 a 17).

Nechápu, jak autorka z obr. 6 usuzuje, že *Podosphaera fusca* je nejbližší taxon ze všech zvolených outgroups. Mohla by komentovat například postavení taxonů Hungary c, Slovakia b, c, d, Estonia b, Netherlands Alkmaar na tomto stromě? (s. 18)

Co znamenají symboly u jmen lokalit ve fylogenetických stromech? (s. 18-20)?

Kde je na zobrazených fylogenetických stromech *E. quercicola*, což autorka zmiňuje na s. 21?

Autorka podle všeho vykonala náročnou laboratorní práci na zajímavém tématu. Vzhledem k formální nedostatkům a nejasnostem v obsahu navrhuji hodnotit předloženou práci v případě kvalitní obhajoby jako velmi dobrou.

V Českých Budějovicích 27. 5. 2010

  
RNDr. Stanislav Mihulka, PhD.

The master thesis by Ms Radka Jungová reports on a study aiming at the identification of fungal species associated with symptoms of oak powdery mildew in several European countries.

This topic is of high relevance in forest pathology since oak powdery mildew is among the most important forest diseases in Europe; more generally powdery mildews is a major group of plant pathogens. Until recently, the identification of causal species was based on morphology but also relied strongly on the host where the symptoms were observed. However, the advent of molecular biology has provided new tools which have allowed to establish phylogenetic relationships between species and dramatically changed common views on species boundaries and host specialization: closely related (conspecific?) lineages have been shown to infect a wide range of species; reciprocally, several morphologically similar but different powdery mildew species have been shown to infect the same host. This applies to powdery mildews found on oaks. The specific objective of the work was therefore to identify species causing oak powdery mildew using one well developed marker : the ITS sequence (part of rDNA cluster) and a new marker developed within the frame of the study, a cytochrome b fragment.

The extensive sampling covering 11 European countries is a strong point of the study (a table summarizing characteristics of the samples is needed: geographic coordinates, date of collection, host, mycelium/chasmothecia, etc...). Previous published studies on oak powdery mildew either dealt with a collection of isolates in a phylogenetic purpose or focused on a particular country for spatial distribution analyses (France). The development of an additional marker to ITS is also highly appropriate: although ITS has become the primary basis of barcoding in fungi (the article by Nilsson et al 2008 should be cited), species identification cannot be based on this sole marker.

However, the study suffers from a number of weak points:

1. The most important issue is a confusion between *Erysiphe alphitoides* sensu lato and *E. alphitoides* sensu stricto (following Takamatsu et al 2007): all along the manuscript, starting with the title, it is never clear whether inter or intra-specific variation is addressed. I acknowledge that the species concept is especially difficult in Erysiphales but since this is the core of the study, this should be clarified (I suggest for the title: Survey of occurrence of oak powdery mildew *Erysiphe alphitoides* sensu lato in Europe using molecular markers). An example of this confusing terminology is given in the introduction section dealing with "identification of *E. alphitoides*": here, it is clear that we are dealing with a complex of cryptic species, but in Figure 5, "*E. alphitoides*" refers to a true species, distinguished from *E. quercicola*, *E. hypophylla*, etc... Next page, please correct "methods for identification to distinguishing *E. alphitoides*" by saying "methods allowing species identification in the *E. alphitoides* complex", etc... (many other examples all along the text). Here the study is focused on and designed for inter-specific variation ; however, the paragraph at the end of section "molecular markers" is another example of confusions between inter and intra specific levels, speaking of "DNA variation within each genus" and then "bottlenecks" and micro-satellites, then coming back to Cox1 and barcoding... There are many examples of awful mixes of intra and inter specific questions throughout the report: at the beginning of the material section, "the European **population** of *E. alphitoides* fungus was analysed for variability and consequent **phylogenetic** relations"; in the are

dealing with inter-specific variation in the complex of cryptic species; “the ITS sequence only reflects **intra-specific variability** within the *Erysiphe alphitoides* s.lat. **species**”, etc, etc.

At the same time, still unresolved question in phylogenies and species identifications should not be oversimplified: it cannot be said for example that “the anamorph stage of *E. alphitoides* is called *Oidium manto* assert species relationships.

2. My second main concern is about the cytochrom b study, especially the interpretation and conclusions which are drawn: from what is presented in methods and results, I think they are going much too far. The mitochondrial genome offer advantages for the development of markers in Fungi but also present serious difficulties due to the presence of many mobile introns (the article by Santamaria et al 2009, BMC Bioinformatics 2009, 10(Suppl 6):S15, could be cited in the introduction). Too little information is given on the design of primers in the methods section. Consequently, results should be interpreted with much caution: indeed they strongly suggest that the primers are not specific and did amplify other fungi. This point should be clarified in the results (“several sequences attached to other fungal taxons were eliminated”: on which basis?). What is the difference between figures 7 and 8? (the legend should be more precise; explain a,b, c, etc....:different clones of the same sample? *E. alphitoides* s.l., etc....)

Interpretations about intra-specific variation, and even more hybridization, strongly need further support.

3. Other minor problems can be cited:
  - Introduction/Literature review: this section is well organized and is a good presentation of the topic but there are some lacks, errors and confusions: e.g. the history of the disease was recently summarized in Mougou et al 2008 (not cited): the first epidemic symptoms were reported in France and not Portugal; the report in Portugal was earlier but not surely identified as *E. alphitoides*; it is dubious whether this report is that of a native species. *E. quercicola* is said as European in some sentences (e.g. page 7), and not reported in Europe a few lines thereafter??
  - The primers described by Heuser & Zimmer 2002 (please correct the reference in text and list) are not *Erysiphe*-specific but designed for ascomycota according to their authors
  - The first part of results (4.1) is indeed a method section: move it before. Conversely, more detail should be given in results. The sentence “the majority of samples were amplicons from *E. alphitoides*”... is not correct. You probably mean: the majority of amplicons showed 100% homology (is that true?) with the ITS of X sequences attributed to *E. alphitoides* in Genbank, etc...
  - The manuscript is written in English, which is laudable, but language editing is needed

Finally, I think that the most sound results of the study are not sufficiently emphasized, especially in the abstract: similarly to what was found in France (Mougou-Haldane et al 2010), *E. alphitoides* sensu stricto appears as the predominant species in a large area of Europe. However, surprisingly, the two other species already recorded in Europe *Phyllactinia* sp and *E. hypophylla* were not found here: this should be discussed. Also, as

in Mougou-Hamdane et al 2010, the recently described species *E. quercicola*, thought to be present only in Asia, is suggested to be present in Europe, based on ITS detection, although in a single sample.

The cytochrom b study deserves more investigation to be used in species delineation or intra-specific variation studies.

As a whole, the study addresses an important topic, with adequate techniques, and provides original results. Although the manuscript could be improved, I recommend the defence of the thesis.

Cestas, May 28<sup>th</sup>, 2010

A handwritten signature in black ink, appearing to read 'Loustau', written over a horizontal line.

Dr Marie-Laure Desprez-Loustau, Senior Scientist