

## REVIEW

Of the member of the dissertation council for the dissertation of Ivan Mikhailovich Pchelin on the topic: MOLECULAR-GENETIC CHARACTERISTICS OF DERMATOPHYTES OF THE GENUS *TRICHOPHYTON* — CAUSES OF ONYCHOMYCOSIS, submitted for the degree of Candidate of Biological sciences in a scientific specialty 1.5.11. Microbiology

Despite the rise of non-dermatophyte skin and nail infections in many areas, dermatophytes are still the main causative agents of superficial fungal infection in most parts of the world. Widespread clonality and unstable morphological traits up until now present challenges to the studies on the taxonomy of the group, currently connected mainly with DNA sequence-based approaches. Molecular genetics data are of constant relevance to the development of diagnostic assays and epidemiological studies. The recent emergence of antifungal drug resistance in dermatophytes calls for further improvements in taxonomic schemes to reflect the structure of genetic and functional diversity.

Mr. Pchelin's thesis presents an original and interesting study of molecular genetic diversity in the genus *Trichophyton*. Its scope includes phylogenetic analysis of the genus with the particular focus on *T. mentagrophytes* / *T. interdigitale* species complex, and the study of the genetic polymorphism in *T. mentagrophytes* / *T. interdigitale* and *T. rubrum* in relation to geographic locations. For *T. rubrum*, the performance of two genotyping techniques was compared. The principal findings were published in well-recognized international journals and represent a substantial contribution to the field. The text contains a number of spelling mistakes and typos, but this would not be a major criticism.

The sections of the manuscript follow the standard order and cover literature review, materials and methods, results and discussion, conclusions and recommended practices. The voluminous appendices contain the list of original GenBank entries and scripts written in the Python language. Introduction explains the significance of the study, including the high estimated prevalence of onychomycosis in the global population and overall paucity of data on the genetic diversity in *Trichophyton* spp. The literature review section starts from a brief description of morphological and physiological traits of *Trichophyton* spp. It is followed by the overview of dermatophyte classification and a summary of studies on genetic diversity of dermatophytes. Given the theoretical and practical significance of the study connected with the global epidemiology of dermatophytosis and population genetics (Page 160), the mentioned topics may need to be discussed in more detail. In accordance with the scope of the thesis, the Materials and Methods section is devoted largely to the description of molecular genetic methods. In part, data analysis was done with originally written scripts.

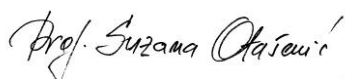
The manuscript has one section for the results and discussion. It starts from the description of fungal phenotypic traits. This part of the work was done with the use of culture collection strains. In four species, the studied samples were limited to just one strain. Given these low numbers and the frequent loss of characteristic morphological traits during repeated culturing, the conclusion on the variability of morphological characters does not seem that convincing. However, it is not mentioned among the main results of the study. The reconstruction of phylogenetic relationships in the genus *Trichophyton* was done with standard methods on a limited number of isolates. The observed division of the species into groups may

be similar to what is known from literature. But, this part of the study has its own rights to exist due to the presence of domestic isolates in the analysis. Probably, the most interesting part of the thesis is devoted to the diversity and geographic distribution of ITS region genotypes in *T. mentagrophytes* and *T. interdigitale*. The discovery of limited ranges in particular genetic lines was followed by several original explanatory hypotheses, including a connection of observed pattern with historical human migrations, possible host shifts in the *T. mentagrophytes* / *T. interdigitale* species complex and climatic factors. To my mind, the description of genetic lines with distinct patterns of geographic distribution would benefit from explicitly stated strict criteria of group delineation. The following subsection describes the analysis of species boundaries. To that end, a number of analyses were performed. A computational analysis of species boundaries in the *T. mentagrophytes* / *T. simii* phylogenetic branch was done with ITS region sequences using ABGD and PTP methods. The question was further explored with phylogenetic networks calculated from three multilocus datasets and phylogenomic trees inferred by two different methods. Here, the main conclusion was related to an intermediate genotype, assigned after the study to *T. mentagrophytes*. The final two subsections deal with the description of the two intraspecific global lineages of *T. rubrum* sensu stricto and a comparative analysis of molecular typing techniques performance.

The Conclusions and Recommended practices sections accurately highlight the most significant results of the study. Despite the concerns raised above, the presented work is novel and matches required standards. It contributes substantially to our understanding of genetic diversity in the genus *Trichophyton*.

Dissertation of Pchelin Ivan Mikhailovich on the topic: "Molecular-genetic characteristics of dermatophytes of the genus *Trichophyton* — causes of onychomycosis" meets the basic requirements established by Order No.11181/1 dd. 19.11.2021 "On the procedure for awarding academic degrees at St. Petersburg State University". The applicant Pchelin Ivan Mikhailovich deserves to be awarded the academic degree of Candidate of Biological Sciences in a scientific speciality 1.5.11. Microbiology. Paragraphs 9 and 11 of the specified Order have not been violated.

Member of the Dissertation Council



Professor Suzana Otašević, MD, PhD

15 April 2023

Niš, Serbia