

Děkanát 1. LF UK  
Oddělení pro vědeckou činnost a zahraniční styky  
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### Ph.D. Thesis Assessment

**Thesis title: "Encystation and life cycle of free-living amoebae of the genus *Acanthamoeba* spp."**

Composing a written report on the research Ph.D. Thesis by RNDr. Eva Bínová, the external reviewer adopted the University guidelines as briefly defined in the request for review and also applied generally accepted PhD thesis assessment criteria.

Since the core of the PhD thesis consists of three papers published in peer reviewed journals of top ranking in the respective clusters (Protist, European Journal of Protistology, Molecular & Biochemical Parasitology), the response to the principal question given to the reviewer is obvious. The evaluated thesis comprises a coherent investigation of the chosen topic based on mastery of appropriate methodology and does meet internationally recognised standards for the conduct and presentation of original research in the field.

The text that accompanies the above mentioned publications allows appreciation of candidate's adequate knowledge of the literature relevant to the subject studied and the field in general, her ability of critical and analytical employment of literature data, and incorporation of her work into that of the *Acanthamoeba* research group.

Of a few critical comments one concerns the title of Thesis. The wording "amoebae of the genus *Acanthamoeba* spp." is rather awkward. It is suggested that the abbreviation "spp." (standing for more than one species, while for a good reason, one only axenized strain of *Acanthamoeba* was actually studied) is superfluous. Without "spp." the title would have still reflect a broad focus of the work, the life cycle and encystation mode characteristics for the assemblage of species described within the genus *Acanthamoeba*. Another option would have been to delete the words "of the genus". Alternatively, the wording "Encystation and life cycle of *Acanthamoeba* amoebae" also appears acceptable.

A certain contradiction was found in the Czech vs. English version of Abstract. The Czech version deals with therapeutic failures in *Acanthamoeba* infections, whereas the 2nd paragraph of the English version deals with therapeutic failures in "amoebiasis". Leaving aside the misspelling in the text ("ameobiasis"), the term amoebiasis (European usage) or amebiasis (mainly American usage) is traditionally reserved for a different nosological entity (i.e., that caused by *Entamoeba histolytica*).

Similarly, as a result of research history, the term Amoebic Gill Disease (AGD) is reserved for *Paramoeba/Neoparamoeba pemaquidensis* infection simply because no other gill disease condition caused by amoebae was known/expected to exist at the time of the term coining. Anyway, the frequent use in the literature of these terms with their specific meanings deserves respect.

Introduction contains portions of text which one can easily find in textbooks on amoeboid organisms (e.g., 1.2.1.) as well as those more closely related to the topic of Thesis and cited from the current literature. There appear some cases of simplification (e.g., in 1.2.1.3., "Acanthamoebae have been recovered from hospitals,") or omission of relationship (in 1.2.2.3., the alleged high mortality rate of cutaneous infections caused by *Acanthamoeba* (73%) is not properly related to AIDS patients in accordance with the reference quoted). These wording shortcomings attract attention of the reader but, in fact, have no essential importance. Unfortunate are also cases of misspelling ("*ProtAcanthamoeba*"; "*Mycobacteria tuberculosis*") and of missing reference (1.3, "cit."). In section 1.4. (as it summarizes data available on encystation and pseudocyst formation, the most important part of Introduction in terms of the Thesis aim and objectives), the reviewer would expect a somewhat more restrained data presentation. In view of the fact that data about pseudocyst duration or resistance, speed of formation, maturation etc. were obtained in experiments under specific artificial conditions, it is recommendable to avoid the attribution of general validity of respective statements, while the artificiality of experimental conditions is always remembered.

In the process starting from recognition of the absence of mannitol in protective reactions of *Acanthamoeba*, through the analysis of trehalose role in stress responses of the same organism, up to the study of DNA content during *Acanthamoeba* encystation and pseudocyst formation, the candidate undoubtedly faced numerous research challenges that could not be explored due to the lack of time. The Oral part of Thesis defence (i) gives the candidate the opportunity to mention some of the challenges, (ii) as she is the individual best informed about gaps in the literature, she might present those in a broader context and identify future directions of research irrespective of her own plans or personal involvement.

#### Recommendation

The assessed Thesis constitutes a significant contribution to the knowledge in the field concerned. I recommend that on the basis of this Thesis, the candidate RNDr. Eva Bínová should be awarded the Ph.D. degree.

Brno, 1 April 2021

  
Iva Dyková, D.V.M., DrSc.