OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student:

Thesis title: Neurological manifestations of SARS-CoV-2 with special focus on anosmia
Supervisor: Mgr. Ján Lopatář, Ph.D.

Referee: Ing. Zuzana Bláhová
Referee`s affiliation: University of South Bohemia in Ceske Budejovice, Faculty of Sciences, Institute of Chemistry and Biochemistry

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	1
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labelling, indication of the units)	0-3	1
Formal requirements – points in total	**21**	13

(2) PRACTICAL REQUIREMENTS		
Clarity and fulfilment of the aims	0-3	1
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work /review	0-3	1
Completeness of the description of the used techniques	0-3	N/A
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	N/A

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[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

The use of up-to-date techniques	0-3	N/A
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	0
Practical requirements – points in total	**18**	**7**

POINTS IN TOTAL (MAX/AWARDED)	**39**	**20**

Comments of the reviewer on the thesis

General comments

The bachelor thesis "Neurological manifestations of SARS-CoV-2 with special focus on anosmia" of Mr. Simon Starker provides an overview of neurological symptoms associated with COVID-19 disease caused by SARS-CoV-2 virus, dives further into reviewing of available information on the loss of smell (anosmia) and finally concludes, which mechanisms might act in manifestation of anosmia in COVID-19 patients. The extent and scope of the thesis is adequate.

The annotation is attractive and clear enough. Accordingly, the student has to explore and categorize neurological symptoms associated with SARS-CoV-2 infection and get in more detail to anosmia as it has increasingly gained attention as a highly predictive marker for COVID-19 disease. Cellular and molecular mechanisms of anosmia are not yet completely understood. Currently, there is an ongoing scientific debate on "what mechanisms do stay behind olfactory disorders caused by SARS-CoV-2". A flood of research evidence on various aspects of Covid-19 disease appears daily. To come out with a theoretical thesis in such a hot topic is highly demanding on searching, reading, understanding and putting in order a substantial amount of information. I highly appreciate the courage of Mr. Simon Starker to write a theoretical thesis, which might rise high expectations of a work full of enthusiasm.

Reading the abstract and introduction, I became disillusioned quickly. These chapters should summarize and introduce to what everything readers will learn from the thesis. Unfortunately, the reader can only deduce the purpose and aims of the thesis as they are not clearly established.

In following chapters I miss the link guiding readers through the text. The text looks like a collage of information. One information appears repeatedly in text as well as in tables. Hence, the reader has to go slowly and carefully through the text to understand the meaning of it. This unreadability might be partly caused by uncertainty in author´s use of English language and/or partly in doubts of which source from those containing similar data to choose for citing, or mostly by the absence of the main idea. On the other hand, there are chapters written relatively clearly, which I have enjoyed as are some parts of the discussion and conclusions, obviously managed with considerable effort. Supporting controversial scenarios of SARS-CoV-2 virus entry into host cells are finally explained and putative coexistence of them concluded. Author´s own idea based on his own experience is also added at the end.

I would like to highlight the fact that the data currently available to this topic are extremely heterogeneous, as preliminary and contradictory results were published very quickly during the pandemic and cannot be regarded as finally clarified. Undoubtedly, it might be challenging for a student at the bachelor degree to read them critically, to keep himself oriented in the huge body of research evidence and finally to be experienced enough to disclose and properly discuss controversies presented. Taking this into account altogether I would judge the stylistic level permissive.

Specific comments:

1. The first paragraph on RNA sequence of SARS-CoV-2 (p.3) and Figure 1 go too much into details and suit better to the chapter *II. Structure and replication of coronaviruses*, where the structure of the genome is explained closely.

2. A paragraph which would state the aims of the thesis is absent at the end of Introduction.

3. The chapter *II. Structure and replication of coronaviruses* does not follow the best logical way of composition: 1. Structure, 2. Replication, 2.1. Replication of coronaviruses, 2.2. Functions of proteins, 2.3. Target proteins in human, 3. Cell entry mechanism, 3.1. Mechanism of cell entry for SARS-CoV, 3.2. Mechanism of cell entry for SARS-CoV-2. I wonder, why Mr. Starker has not followed a more logic structure, which would be for example: 1. Structure, 1.1. SARS-CoV-2 virus particle, 1.2. SARS-CoV-2 genome, 2. Entry of SARS-CoV-2, 3. Viral gene expression and RNA synthesis, 4. SARS-CoV-2 - host interaction and host response.

4. In my opinion, Figure 6 titled: "Daily new confirmed COVID-19 deaths per million people in five countries selected.", seems not much relevant to the topic on neurological manifestations of SARS-CoV-2 with special focus on anosmia, as it does not show how many of the deaths could be related to a neurological disorder.

5. Tables 2, 3, 4 and 6 deal with symptoms of the COVID-19 disease, however, do not bring any information to reader interestingly. Moreover, Table 4 and the following text have inconsistent segmentation. The information on psychiatric diseases makes the chapter confusing. The thesis could benefit from a nice scheme or picture demonstrating neurological symptoms of COVID-19 and using pie charts when showing further data. Besides the range of prevalence of symptoms manifested in the population, further categorization could be performed, for example by presentation (early/late in illness), severity (severe/non-severe), age or location. More than one single publication could serve as data source to reach higher informative value.
Table 5 does not deal with symptoms of the COVID-19 disease, hence it is irrelevant and should be left out as well as all the information on psychiatric problems which are not caused by COVID-19 disease directly and can be related to socioeconomic stressors.

6. In the chapter *II. Neurological symptoms* appears the statement that "according to the current situation, it is expected to have in between 24,818 and 132,976 patients with CNS complications and in between 33, 096 and 106, 383 patients with PNS complications (p. 14)", but there is not specified when in the future or in what part of the world that might happen.

7. Another example of data, which do not contribute much to the topic of the thesis is on page 27. There are enumerated viruses which are known to invade peripheral olfactory structures (Polio, Indiana strain of wild-type vesicular stomatis, Rebies, Herpes simplex types 1 and 2, Mouse hepatitis virus, Herpes suis, Borna disease, Canine distemper viruses). None of these viruses is explained further and no valuable information with regard to COVID-19 manifestation is brought to reader.

8. Figure 11 (p. 28) is irrelevant. I do not understand the reason for presenting a graph showing the ratio of people suffering from chronic decreased sense of smell which is not related to COVID-19 disease.

9. Similarly, paragraphs *Toxic chemicals and nanoparticles, Head trauma, Other disorders* as well as Figure 12 have nothing to do with neurological symptoms of COVID-19 and absolutely do not

reflect what the annotation of this thesis aims at.

10. The sentence "not being able to smell – anosmia- is a dysfunction of the olfactory system and can have a tremendous impact on people´s quality or even affect their health situation" (p. 30) is excessive, as this information appears already on p. 3 and p. 15.

11. Since anosmia is the main theme of the thesis and numerous scientific publications have been published very recently targeting this topic, I would recommend to add a table listing possibly all available reviews and meta-analyses which give COVID-19 and anosmia into relation.

12. Chapters *V. 1. An insight from animal models* and the subsequent chapter *2. Possible mechanism for the non-transient anosmia in COVID-19* should be merged and thoroughly rewritten since they are incomprehensible.

13. In the *Bibliography* there are references missing (Ellul et al. 2020, Saussez et al. 2020), two without any citing in the text (Bulfamante et al. 2020, Cocco et al. 2020), in one reference, there is mentioned wrong year of publishing (Wang et al. 2016).

Conclusion:

Despite the substantial reservations I recommend the thesis for the defence.

Reviewer´s questions for the thesis defence:

1. In the Introduction, you have mentioned the existence of other coronaviruses which have been known for long time and have caused several other epidemics in the past. SARS-CoV-2 is the new coronavirus emerged. At this place, I would like to ask: What is the main difference between SARS-CoV-2 and the other known coronaviruses, what makes him accelerating pandemic more effective?
2. What do you think is the main reason for current big research interested into anosmia?
3. In the *chapter 1. Structure* you have finished the 1 paragraph by the sentence: "RNA compared to DNA viruses have a much higher mutation rate." without any further explanation. Do you know why it is so? Does this feature play any role in the treatment of COVID-19?
4. Patients with moderate to severe form of COVID-19 disease have less olfactory involvement. It is not known to date, why it is so. Do you have any hypothesis?

In Ceske Budejovice 20th of January, 2021