The thesis entitled „Symmetry of green algal cells and their colonies resulting from adaptive phenotypic plasticity under planktonic life history" aims to study the association between phenotypic plasticity (operationalised as asymmetry measured with tools of 2D geometric morphometrics) and environmental conditions in green algal species *Desmodesmus communis*. Previous evidence exists of a relationship between planktonic life history and phenotypic plasticity (asymmetry).

The candidate sets three axes of symmetry: vertical, horizontal, and transversal. Initially, the last one sounded the most surprising to me. Despite persisting doubts, it seems this was a justified decision (one must focus on the landmarks' position, not the coenobium's outline). The following three conditions were selected to describe the living environment of coenobia: mixed (the water body moves/mixes permanently), stationary-mixed (the water body mixes only in some parts of the day), and stationary (no mixing of the water body). The thesis mainly focuses on the polyphenism of coenobia (pseudo colonies of *D. communis*), "a subcategory of developmental plasticity, where different alternative phenotypes arise from one genotype in response to certain environmental conditions (Suzuki *et al.*, 2020)" and tries to create conditions that result in such a phenotype change (asymmetry & size). The candidate expects more pronounced asymmetry in the coenobia living in still water. On top of testing whether different conditions account for different levels of asymmetry, the study aimed to explore which of the three axes of symmetry is the most determinant (in the morphometric/morphologic sense) of the shape of D. communis with respect to asymmetry.

According to the introduction, many environmental factors account for the shape of coenobia – predators, water dynamics, temperature, and other abiotic stressors (pH, kairomones, heavy metals), etc. It is well structured and written in good English, the sentences are not overly long, and I think I only spotted one typeset error. Also, the studied problem is exposed well; however, the end of the introduction lacks a detailed justification of the methods and characterisation of the experimental design. I am aware of the section in which goals are listed. Nevertheless, repeating important goals (plus how the selected methods can achieve these) would make sense, too.

Out of the four "strains" (specimens taken from different, geographically distant habitats and considered genetically isolated), one was kept in a laboratory run by human researchers for fifty years. The others were from a pond, river, and a de facto natural lake. Therefore, the study is quite representative concerning the ecological variance of central European habitats. What would otherwise be a clear advantage was made less clearly advantageous by the decision to analyse each strain separately.

The candidate put selected single-cell specimens in the three experimental (mixing) conditions and let them grow. Subsequently, microphotographs of four-celled coenobia were taken; they were cropped and rotated in the GIMP program (version 2.10.38) so that each photo was "as similar to each other as possible." This was probably done just for the convenience of the analysis: GMM in Geomorph would run the GPA, resizing, aligning, and rotating the specimens automatically (unless it makes sense to orient and locate the specimens before the GPA, which is not mentioned). Subsequently, the candidate "created an empty TPS file in tpsUtil and then imported the landmark coordinate data into the file during digitisation." While this is a correct description, it is uneasy to grasp for anybody outside the field – it would be enough to write "specimens were landmarked in tpsDig" (or is that the case?). On the contrary to this detailed piece, I find the overall depth and scope of the methods description at this stage on the bottom of page 12 (18) inadequate.

The study relies on calculating asymmetry by rotating specimens. The strains, the condition ("environment"), and the presence or absence of spines in the analysed landmark-denoted shape are considered, and asymmetry is calculated separately for each of the listed. I supposed my problems with understanding the details of the method resulted from my decision to study faces instead of green algae. When I visited the articles in the thesis (e.g., Savriama, Y., Neustupa, J., & Klingenberg,

C., 2010), I got the information I lacked (the sole exception will hopefully be addressed during the defence; see question 6 below).

The Results section is complex, lengthy, and exhaustive. The candidate managed to acquire advanced methods of geometric morphometrics: characterising asymmetry of objects that possess more than one axis of symmetry, identifying principal components of the within-sample shape variance, visualising them and interpreting with respect to the research question. Other acquired skills that would otherwise be "enough for a thesis", like cultivating the specimens under controlled experimentally induced conditions, shooting and editing microphotography and analysing the collected data, interpreting the results and reporting them, suddenly appear as granted and natural (which is not the case, though). Nonetheless, the presentation of the results is sometimes problematic. The first issue relates to the language used to describe the results: some parts of the report use very peculiar terms and put the standard "statistical notation" into a new light by integrating statistical terms into unconventional phrases:

p 13 (19): "Nonoverlapping confidence intervals indicated that the studied datasets were indeed significantly different in the degree of asymmetry." There is a problem with the term "significantly". However, I like the analysis. Formal statistical tests shall not usurp the primacy of deciding on hypotheses unless all conditions necessary for such tests not to be misleading are kept. However, CI is a descriptive statistic, not a statistical test. The conclusion should somehow avoid the term suggesting there is a [statistically] significant difference. Maybe I am criticising you for a mistake that is just an outcome of different schools of statistics – and the tools used by the candidate are from outside the "moralist NHST". I suppose this point will be reconciled during the defence.

Above, I tried to be grateful, but the next sentence (p 14 [20]) is just mixing up tests' and (again) descriptive statistics: "The **significance** of the correlation analysis was then **measured** using **Pearson's r** (Nettleton, 2014)." Significance is decided based on the p-value, according to test statistics, and preset conditions (alpha level, meeting conditions of the test like independence of observations, etc.). It is not measured. The test results are somehow indicative, but we are always free to decide on what they mean! There are also other descriptions that I find hard to follow (e.g. "For both the stationary and mixed treatments, the mean, median, and their confidence intervals achieved correspondingly high values." – does this mean that the asymmetry was more pronounced and, given the CI, convincingly so?

p 14 (20): "This test recalculates a particular statistic several times, and if the results are significant, this recalculation should not affect them." Please explain.
p 15 (21): "Negative Pearson's p value indicated a negative correlation between two variables (Nettleton, 2014). This implies that the lower the Pearson's p value, the more asymmetric the objects were." Potentially, you are talking about a coefficient, not a p-value, a "probability" of the observed test result (typically a quantile), assuming H0 is correct.

In the section "3.2. Relationship between the size of the coenobium and its asymmetry", there are also some parts that report the numerous results in statistically problematic ways: "Strains B and D had the lowest Pearson's p value…" The p-value is unrelated to Pearson's correlation; it is a probability based on test statistics, see above. And lastly, are sentences like "However, some values were noticeably close to zero and so could not be classified as significant" sophisticated attempts to provoke a frequentist statistics fundamentalist or a result of misunderstanding the nature of NHST?

The last section of the results, "3.3. Analysis of symmetric and asymmetric variability…" reports the results of PCA analysis and provides "a more detailed description of the symmetric and asymmetric variability among the four strains" as it identifies PCs of shape and asymmetry change with size change. This section is, in my opinion, the best part of the thesis; I find the PCAs ideal for characterising (and visualising) the association between ecological conditions, size, and shape.

Nonetheless, the problem of splitting the analysis into four independent parts persists. The cognitive (or memory) load for/of the reader is quite demanding.

It is a general point of this review that the data should have been pooled and analysed together, as this would help to distinguish (interesting) similarities from (potentially interesting) phenomena in individual strains (even though strain A should always be left out). Potential differences could then be immediately assigned to ecological conditions (which the candidate did only later in the discussion section). I hope, and suppose, this will be fixed for the data publication. We see the same analysis four times (a series of analyses) without accounting for that using hierarchical tests or meta-analytic methods (or a table summarising the results). Moreover, this need not be an either… or… setting. The thesis allows enough space for the candidate to report the results using both approaches – separately for each strain and together (the thesis now has just 67 pages).

Numerous figures and tables accompany the results section. This is a desired state; yet again, some aspects of the tables and figures are problematic. Some tables and figures (Table 2, Figure 5) have a very small resolution. It is an unfortunate issue, given the overall quality of the thesis and the amount of work behind it. Is it another product of PDF/A illness SIS still suffers from? Back then, students were forced to render PDF/A2, which was sometimes hard to get using MS Office. Some online apps do the job by turning all the problematic parts into low-resolution images. Sharp vectors become blurry, and textures collapse into rugged bitmaps. Is this the case here? Moreover, Figure 5 and the related description switch the order of the treatments. It is a negligible mistake for a thesis but one that would be pointed out by a reviewer of a manuscript submitted to a journal. Moreover, based on the plots in F9-F12, I cannot say I see/spot any substantial difference between the strains' asymmetry based on treatments. There is no notation to suggest what I see in the diagrams (perhaps standard ones) and whether I should consider the differences to support the alternative hypothesis that there is a systematic, non-random difference between treatments, potentially across the strains.

The **discussion** sums up the results in writing: The thesis fulfils its overall goals. It reveals that mixed and partially mixed environments make the strains more asymmetrical (this was not originally assumed), and this variance is particularly pronounced for spines, not the coenobium analysed without spines. Small coenobia that have recently separated from a mother coenobium possess a relatively pronounced level of asymmetry. The lowest asymmetry was observed in stationary treatment, which agrees with the hypothesis that symmetric spines lead to slower sinking in such an environment. The coenobia stay in the euphotic zone for a longer time and prosper.

The thesis further concludes that the symmetry of spines may compensate for the asymmetry of the core of the coenobium. Desmodesmus is specific concerning its asymmetry on the transversal axis, which has not been observed in other species/lineages of green algae. Moreover, the thesis also demonstrates that asymmetry rises as a function of the size of the coenobium. The discussion also highlights that strain A, kept in a laboratory, systematically differs from the strains collected outside of it. This leads to the question of what the analysis would look like if only strains B-D were included and if they were analysed in a single analysis (perhaps with a multi-level structure). It also provokes the reader to challenge the results of other studies based on laboratory strains exclusively.

Except for section 4.3., the study does not discuss the results with respect to other Chlorophyceae and, in general, other living species. This is a good decision given the specific planes of symmetry in coenobium, which complicates the generalisability of the results. In sum, the spines adapt to changes in environmental conditions and influence the final shape of the coenobium to the largest degree.

These are relevant and interesting results, yet the thesis does not provide a clear summary of the results section, partly due to the decision to analyse each strain and, within it, each treatment separately. While this issue is partly addressed in the discussion section, checking whether the results are discussed correctly presents a relatively demanding task for the reader. The methods are also sometimes described insufficiently (however, this is my opinion, as a person who is not a trained algologist). Together, this makes the thesis harder to follow. For publishing the results in a peer-reviewed scientific journal (which is recommended), I suggest using multi-level (or at least meta-analytic) methods so that the results better expose trends shared (or otherwise) across the strains. While this might be considered a problem, it is not a serious one for a Master's diploma thesis.

Conversely, the candidate demonstrates their skills in developing experimental design and obtaining relevant information from the scientific literature. As it is obvious, the candidate also acquires skills in the methods needed for the appropriate data collection, collects the data, analyses them and presents them in good English (a reader is suggested to compare my English in this review with the English of the thesis; I suppose the winner would be the thesis itself). While this aspect of the thesis (language superiority) may be partly due to using large language models as editing services, this is not a criticism. I was told a myth that once it was considered a sign of good taste to get the thesis language checked by an independent editor before submission.

As for the formal rating, if the defence answers my questions (please see the last sheet) and presents the results in a more concise manner, the grade "výborně" shall be the case.

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Vojtěch Fiala, v.r.

List of questions:

1) Can you please sum up the results regarding a systematic association between the level of asymmetry, conditions in which the strain lived in nature and in which it was cultivated (potential interactions)? What I mean is a single table showing how the strains reacted to experimental conditions.

2) The candidate writes: “Geometric morphometrics is a particularly valuable tool (…), since it can intuitively describe changes in the shape (phenotype) of an organism under different conditions.” What does the term “intuitive description” mean? Is this somehow related to the concept of a unique, species-specific organic form that a layout of landmarks can represent?

3) The candidate frequently mentions “phenotypic plasticity (…) in response to the presence of predators”. Let us, therefore, suppose that predators are one of the key ecological conditions. How would you characterise the association between predator pressure and other environmental stressors (water dynamic characteristics of the environment) when it comes to the shape and size of the coenobium (i.e., we know how the predator works, how the mixing works – but how these “factors” interact)?

4) “Furthermore, my analyses showed the highest asymmetry with regards to the transversal axis.” Can you provide a brief theoretical analysis of sources of asymmetry on the three axes? In my opinion the asymmetry along transversal axis would be more pronounced than on the other two axes, based purely on geometric conditions (i.e., imagine folding a quadrilateral structure diagonally).

5) A2: “In the second analysis I wanted to find out what the relationship is between the size of the coenobium and its asymmetry.” (…) “The slope a is a line intersecting the average of all measured data and therefore represents a linear model of the relationship.” Can you explain it further?

6) The candidate acquired the method of Savriama et al. (2010) for the analysis of symmetry. Nonetheless, to what I see, the method applies for a “symmetric structure with two perpendicular axes of bilateral symmetry”. Can you please explain the consequences of also considering the third axis of asymmetry (transversal)? Excuse me, I do not understand how this method can be used to characterise the asymmetry along the transversal bilateral symmetry axis.