



## **Opponent's review of Ph.D. Thesis**

Thesis title: **Evolutionary pattern and mechanism of environmental sex determination in reptiles**

Thesis author: **Mgr. Barbora Straková**

Supervisor: **prof. Mgr. Lukáš Kratochvíl, Ph.D.**

The presented Ph.D. thesis addresses several aspects of the evolution of environmental sex determination (ESD) in squamate reptiles and particularly in geckos. The work encompasses four published papers (one review and three original research articles) in respected journals, including one paper in the prestigious journal *Current Biology*. The candidate is a first author in two publications and it is worth noting that the number of authors in the papers ranges only from three to seven (i.e. the candidate is never lost in the sea of multiple authors). This amount of published work clearly fulfills the doctoral study program requirements.

### **Thesis objectives and outputs**

One of the main objectives of the thesis was to contribute to untangle the issue of how many geckotan species determine sex via ESD. As the author eloquently discusses, still in the recent past, it had been commonly assumed that majority of reptilian species utilize ESD. But since then, particularly the research group to which the author belongs, made a substantial contribution to understanding that most of previously assumed ESD species (especially in squamate reptiles) are actually triggering the sex-determining pathway by sex chromosomes (i.e. they employ genotypic sex determination). An important note raised by the author is that while novel genomic tools allowed much smoother identification of sex chromosomes in a wide range of species, obtaining adequate experimental support to prove or dispute the ESD mode remains still a difficult and laborious task which requires sufficiently large sampling and wide temperature conditions to be tested, along with other issues to be solved. Hence, I appreciate that the candidate had a courage to choose this thorny road for her Ph.D. project and, moreover, she indeed achieved highly valuable scientific results. By bringing to light a satisfactory evidence for ESD in five gecko species (papers in Chapters 2+3) the author and her team doubled the confirmed gecko species with ESD. Of note, despite the knowledge of exceptional variability of sex determination systems in geckos, with frequent turnovers among them, the author clarifies that the sex-determination mode still has not been studied in an overwhelming majority of gecko species, which leaves the opened door for quite extensive research in the future. Furthermore, in the chronologically first publication (from 2020) which is the review paper, the authors raised a hypothesis about the ancestral state of sex determination in amniotes, thus expanding the earlier studies of the team (e.g. Johnson Pokorná and Kratochvíl 2016, *Biol. Rev.*; doi: 10.1111/brv.12156). Last major scientific output of the thesis is from the study published in *Current Biology* (Tezak et al. 2023; Chapter 4) which reports an unexpected finding that temperature affects the number of primordial germ cells (GCs) in the embryonic gonad. It is the first evidence that GC number influences sex determination in amniotes. Author and colleagues suggest that high GC number during female development may confer adaptive advantage for TSD.

### **Thesis formal structure and quality**

The thesis is conceived as a compilation of thematically related papers. According to the candidate's CV, there is only one another (thematically unrelated) co-authored paper which was not included in the thesis

and thus it seems (perhaps seemingly) that the author was fully concentrated on the main tasks of the thesis and did not distract herself much by the side-projects. I was a bit surprised that the thesis was delivered in two separate pdfs, one of which includes Chapters 1-4 representing each individual article, and the second providing the English-written thesis text (34 pages) with a rather typical structure such as i) list of publications including the specified contribution of the candidate to the work, ii) a literature overview (which is, however, not presented under such main title and thus we directly jump into the set of sections informing about the studied problematics), iii) Conclusions and further perspectives, and iv) list of references. I somewhat missed a clear recapitulation of the aims and main take-home messages of the individual four published articles. I'm also used to seeing a list of methods the author employed during the experimental part, but given the very specific nature of the experiments performed here (e.g. testing the effects of incubation temperatures on sex ratio and other parameters) this is perhaps not a relevant remark.

The thesis reads well, with minimum typographical errors. I must admit I enjoyed the writing style not only in the thesis text (phrases such as „farfetched idea“, „amusingly enough“ or „will rise a few eyebrows“) but also in the articles. One example where I (hopefully not by mistake) enjoyed a decent sense of humor, can be found in Peš et al. 2024 (paper of Chapter 3): „Nevertheless, this view remains somewhat isolated, perhaps because randomness is difficult to study..“

The main text contains three figures, two of which nicely summarize the current knowledge about sex-determination modes in amniotes and geckos. 119 literature resources were cited and here I found some discrepancies between the bibliography and in-text citations. Given the overall quality of the work I do not consider them as important and rather I list the identified deficiencies below for the author's needs:

References present in the text but missing in the List of references: Anderson and Oldham 1988 (maybe substituted by Anderson 1993?), Ewert and Nelson 1991, Holleley et al. 2015, Inamdar et al. 2012, Lin et al. 2018, Liu et al. 2024

Koopman et al. 1991 is cited as 1990 in the text.

Two papers by Kratochvíl et al. 2021 are cited (not distinguished as „2021a“ and „2021b“)

**Here I have several specific remarks, suggestions and questions which I would like to ask to be addressed during the thesis defense:**

Page 13: "Viviparous skink with assumed TSD, *Eulamprus tympanum*, shares identical sex chromosomes as the rest of the skinks (Kostmann et al., 2021) and, therefore, is not an exception to this rule."

I would like to point out that particularly in fishes, out of 77 species with reported TSD, 34 possess also sex chromosomes. Therefore, may the presence of sex chromosomes indeed automatically rule out the possible role of TSD in all reptile species (?)

Page 17: I was a bit surprised that for such a frequent statement that fishes exhibit large variability of sex determination systems, the chosen underlying reference was Rajendiran et al. 2021. By using the language of the author, this „raised my eyebrows“. On one hand, I thank to the author for enriching my papers collection. On the other hand, I would like to recommend some perhaps more robust reviews for the future reading: Devlin and Nagahama 2002 (doi: 10.1016/S0044-8486(02)00057-1); Heule et al. 2014 (doi: 10.1534/genetics.114.161158); Godwin and Roberts 2018 (doi:10.1007/978-3-319-94139-4\_11); Guiguen et al. 2019 (doi: 10.1002/9781119127291.ch2); Shen and Wang 2019 (doi: 10.1002/9781119127291.ch4); Nagahama et al. 2021 (doi: 10.1152/physrev.00044.2019).

Page 18: cited Whiteley et al. 2021 (not clear which one from 2021a-c).

Page 20: I appreciate the explanation of the molecular mechanism of TSD in the turtle *Trachemys scripta* two times differently on this page. It is not an easy information to digest, therefore, although repetitive, it was helpful.

Page 20: SR proteins not explained.

Page 23: A comment to the sentence: „All of this complicates the transition from GSD to ESD.“: But it seems not so complicated in homogametic sex which does not carry the master sex-determining gene.

### Questions:

- 1) Since the publications have passed a demanding review process, it is not my place to subject them to further critical analysis. But I would like to ask what was the most discussed or problematic point (if any) during the reviewing process of these four papers?
- 2) In the Abstract and at several other places (including in the publications) the author states that molecular mechanisms of ESD remain unexplored in squamate reptiles which hinders robust conclusions about ESD homology in amniotes. Later on page 26 there is a note that a further logical step would be to investigate the causal connection between genes and proposed models through manipulatory experiments. I would like to ask if the author and/or whole research group already has some more specific strategy how to proceed in this in future. If yes (and even if no), does the author also consider to expand the research scope to fish species with ESD (e.g. via collaborative work) in order to achieve more generalized view on this matter?
- 3) The author nicely explained three modes how the continually changing temperature may influence male vs. female ratio (FM, MF, FMF). Could the author comment on how these modes (and if there is any another mode) are distributed in fishes with TSD? By acknowledging there is indeed a vast literature on fish sex determination to be searched in a restricted time, I would like to give a hint that the relevant information has been summarized in a recent review paper.
- 4) The author focuses in detail on ESD in reptiles driven by temperature and stress-related hormones. But the potential triggers (ambient conditions, stress factors) might be more diverse in fishes. I suggest to elaborate this issue during the thesis defense.
- 5) In the review paper Straková et al. (2020) the authors state in Conclusions that ESD shares several important aspects with sequential hermaphroditism occurring in fishes. Has there been any research development during the past four years which would further support (or dispute) this idea?

**To conclude**, the candidate achieved interesting and scientifically sound results in the field of reptilian sex determination and demonstrated the ability to conduct creative scientific work. I recommend this thesis to be accepted as Ph.D. thesis and after addressing raised points and the successful thesis defense I suggest to confer a scientific degree Ph.D.

In Liběchov, 3.9.2024

Mgr. Alexandr Sember, Ph.D.