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STATEMENT

on a dissertation for awarding the scientific and educational degree "Doctor" in the scientific specialty "Ecology" to Antoniya Yurieva Hubancheva,

full-time doctoral student at IBER-BAS with the topic

"Sound Based Predator-Prey Interactions Between European Bats and Bush-Crickets"

by Dr. Rachel Page, Smithsonian Tropical Research Institute, Panama

In this excellent dissertation, Antoniya Yurieva Hubancheva demonstrates expertise in multiple distinct methodologies – from the molecular analysis required for DNA metabarcoding, to the intensive fieldwork required to track bats in nature, to acoustic playbacks and recordings of sexually signaling prey in captivity. Antoniya combines these distinct methodological approaches to shed light on the foraging strategies used by predators, and the tactics their prey use to avoid them. The result is a strong thesis that offers important contributions to the field.

In her first chapter, Antoniya uses DNA metabarcoding to document the diets of 60 greater mouse-eared bats (*Myotis myotis*) and 53 lesser mouse-eared bats (*Myotis blythii*). Results concur with what is known about these predatory bat species to date: crickets are more prevalent in the diet of *M. myotis* while and bush-crickets are more common in the diet of *M. blythii*, with the relative read abundance of carabid beetles differing significantly between the two bat species. The diets of these bats also include endo- and ecto-parasites, offering the potential for this dataset to be used to examine multi-tropic community interactions. This chapter of Antoniya's dissertation is the most descriptive and the least hypothesis-driven of the three, but represents substantial effort and demonstrates that Antoniya has mastered techniques required for DNA metabarcoding, an important molecular approach that has become increasingly useful in shedding light on previously cryptic ecological interactions.

In her second chapter, Antoniya combines DNA metabarcoding with tracking free-flying bats in nature to examine foraging strategies in the wild. This exciting approach uses miniature onboard microphones to show that even though aerial hawking is a more successful strategy for *M. myotis* bats, with more prey captures per capture attempts, aerial prey is smaller and less energetically rewarding. Antoniya shows that *M. myotis* more often choose the higher risk – higher reward strategy of gleaning larger, more energetically profitable prey from the ground, even though they have higher failure rates with this foraging strategy. This well-designed study cleverly combines data from multiple sources (molecular analysis of bat feces, acoustic data from onboard microphones attached to free-flying bats, ground truthing with recordings of captive bats, analysis of three-dimensional movement patterns) to shed light on the hunting strategies bats use in nature. While numerous studies have investigated bat foraging behavior in controlled conditions in captivity, captive tests offer relatively simple foraging tasks, without the complexity of shifting, complex real-world conditions. Antoniya's study is groundbreaking for this reason, and sheds light onto the shifting tradeoffs bats must actually balance in nature.

In this tracking chapter, the text states that '…after rain, the rustling sounds of walking arthropods on leaves are more difficult for bats to detect potentially halving the detection range'. The sounds generated by ground-walking arthropods would indeed be more difficult to detect in rain, making terrestrial prey harder to capture. Was it raining on any of the recording nights, and was this followed by the predicted shift in foraging behavior (a reduction in gleaning, and increase in aerial hawking)? Wouldn't aerial prey be more difficult to capture in rain as well, given the echoacoustic clutter produced by raindrops? In future work, it would be fruitful to quantify external environmental factors in concert with tracking data. Do weather conditions such as the onset of rain or wind coincide in a shift to aerial hunting? Quantifying aerial insect abundance is also key. Do ephemerally abundant insect swarms drive a shift to aerial capture behavior?

After investigating predator foraging behavior in detail, in the third and final chapter of the dissertation focuses on prey. Here Antoniya investigates the behavioral strategies sexually advertising bush-crickets use to minimize detection by eavesdropping predators. In this elegantly designed experiment, Antoniya compares the courtship behavior of young versus old male bush-crickets in response to acoustic stimuli that simulate different levels of

predation threat by echolocating bats. Male bush-crickets stop producing their courtship songs with greater frequency and for longer periods of time when predation threat levels are high. Interestingly, the study finds age differences in these behavioral responses. Young males are cautious in their signaling: following a simulated predator encounter, they are silent for a longer time. Old males, in contrast, are less acoustically cautious, and show less reduction in signaling in the face of simulated predator risk than their younger counterparts.

The sample size for this experiment is small (n=11 bush-crickets in total). Of the 11 bush-crickets that were originally tested when 'young', only seven survived in captivity to 'old' age, allowing for a second round of testing. As such, four new bush-crickets were brought into the experiment for the old age tests. While it's not ideal that the group of 'old' crickets tested in this experiment experienced highly different circumstances (seven individuals spent their aging period (July to September); four individuals spent this period of time in the wild). It would be interesting to see if the antipredator response in these two groups differed, or if the propensity to keep signaling, even in the fact of predator risk, is common in these old individuals, despite their difference in experience during the aging process. Even with this caveat, this is an excellent study that offers new insight into the signaling strategies of sexually advertising prey in the face of eavesdropping predators, and how these strategies shift over the lifetime of the signaler.

All three of these chapters are in manuscript form. The first two have already been published. Antoniya is the first author on the DNA metabarcoding study, which was published in the journal *Metabarcoding and Metagenomics* in 2023. Antoniya is the second author on the high risk / high reward *Myotis myotis* tracking study which was published in *eLife*, also in 2023. The final chapter, on lifelong flexibility in bush-cricket courtship signals, has not yet been published, but is already in manuscript form, and is posted on *Authorea* as a preprint. This manuscript is in excellent form and I expect will likewise be published soon.

In sum, this is an excellent dissertation. Given the strength of these three studies, including intensive fieldwork, analysis and writeup, I strongly recommend awarding **Antoniya Yurieva Hubancheva** the scientific and educational degree "Doctor".