

## PhD Thesis Review Report

**Candidate's name and surname: Javier Oñate Casado** .....  
**PhD Thesis Title: Patterns of song variation in migratory and sedentary birds: a comparison of two sister pipit species** .....  
**Thesis Supervisor: Tereza Petruskova** .....  
**Reviewer: Paweł Szymański** .....

### **1. The topic of the dissertation**

While Chapter 1, "Guess who? Evaluating individual acoustic monitoring for males and females of the Tawny Pipit, a migratory passerine bird with a simple song," and Chapter 3, "When individuality obscures geographic song variation: a comparison of two passerine sister species with different migratory strategies," are closely aligned with the core theme of the thesis, "Patterns of song variation in migratory and sedentary birds: a comparison of two sister pipit species," Chapter 2, titled "An experience to remember: lifelong effects of playback-based trapping on behaviour of a migratory passerine bird," is somewhat less directly related to the main focus. Although this chapter provides valuable insights into the broader context of avian behaviour, it primarily explores the long-term effects of playback-based trapping on bird behaviour. As such, it diverges from the thesis's central theme of song variation patterns by concentrating more on behavioural impacts related to research methodologies rather than directly addressing song variation in species with different migratory strategies.

### **2. The candidate's knowledge**

The dissertation clearly demonstrates the candidate's solid theoretical knowledge in the discipline of bioacoustics. The research is grounded in well-established theoretical frameworks and effectively shows a comprehensive grasp of evolutionary mechanisms, species behaviour, and geographic variation. Furthermore, the literature review throughout the thesis demonstrates the candidate's ability to critically assess and apply relevant biological information within the field.

### **3. Independence of the candidate**

To begin with, I really appreciate the effort invested in the fieldwork, particularly given the challenges of conducting research in the warm and sunny climates of Madeira and the Canary Islands. This is especially noteworthy considering it was winter in Poland and Czechia at the time.

On a more serious note... Despite the collaborative nature of the papers included in the thesis, it is evident that the PhD candidate played a leading role in the research process. The candidate's significant contributions are demonstrated through his involvement in designing the study, collecting acoustic data, conducting key analyses, interpreting the results, and preparing the manuscripts, all of which ensure the depth of the research presented in this thesis. Moreover, the candidate's skill to integrate contributions from co-authors highlights his organizational strengths and ability to maintain focus on the research objectives.

#### **4. Originality of the dissertation**

##### Chapter 1

The paper published in the *Journal of Ornithology* reinforces earlier findings that male Tawny Pipit songs remain temporally stable both within and across seasons. Significantly, it also provides new insights into female singing by demonstrating for the first time that female Tawny Pipits produce individually distinct song types. Furthermore, the study reveals that males and females are indistinguishable by ear in the field and exhibit no structural differences between sexes. These results advance the development of Individual Acoustic Monitoring (IAM) methods, a novel approach that could be utilized to study the behaviour and ecology of this species.

##### Chapter 2

While a few previous studies have already demonstrated that using playback to lure birds into mist nets, followed by handling, can influence their future behaviour, the paper published in *Animal Behaviour* is the first to show that a short-lived passerine can retain this experience and alter its behaviour to avoid it for a period that matches or surpasses its expected lifespan in the wild. This finding is novel and has significant implications for any research involving playback experiments on birds that have been individually marked in prior studies.

##### Chapter 3

This manuscript presents the first in-depth comparison of the songs of Tawny and Berthelot's Pipits, examining geographic variation through extensive acoustic sampling across multiple locations within their ranges. Additionally, it highlights the presence of individual vocal signatures in Berthelot's Pipit, offering new insights into vocal distinctiveness both within this genus and beyond.

#### **5. Questions and/or criticisms to which the Reviewer expects the candidate to respond during the defence**

General comments/questions on:

a) Chapter 1

- Methods. *"The population has been intensively monitored for several years (most intensively between 2015 and 2017), and the birds captured during the survey were equipped with a ring with a unique alphanumeric code allowing their visual identification from a distance."*

It would be helpful to understand the level of site fidelity/return rate within the population.

- Methods. *"For those individuals that were ringed at the time of recording, we additionally took note of their ring code, if visible."*

At any point, was the sex of individuals identified as females during ringing confirmed through methods such as genetic analysis? How were individuals sexed if neither males nor females had colour rings?

- Results. *"In most cases, song renditions of the same singing individuals (whether male or female) were alike, regardless of whether these originated from the same recording, from recordings obtained in different parts of the same season, or from different years (Figs. 1, S2)."*

Would the probability of classifying unknown songs into a certain group (individual) change if a larger number of song renditions from a single individual were analysed? How were the specific song renditions (typically limited to three) selected if you recorded more? The same question applies to the Berthelot's Pipit data from Chapter 3.

- Results. *"Identification based on song recordings was consistent with the observation of ring codes, indicating that when analysing the spectrograms with care, we could distinguish individuals of both sexes with a considerably high level of certainty."*  
How many songs need to be recorded to accurately identify an individual solely through visual inspection of the spectrogram?

General comment: I would be keen to see the results of a Mantel test that examines the correlation between song dissimilarities and geographical distances between individuals. While I understand that individuals typically have distinct song types in their repertoires, leading to minimal or no repertoire sharing, it would still be intriguing to determine whether neighbouring individuals and those farther apart share any common elements in their songs. The same consideration applies to Berthelot's Pipit.

#### b) Chapter 2

- Methods. *"These variables (except for the total time of interest) were included in a principal component analysis (PCA) to summarize the overall aggressive reaction of males to the playback stimulus (Fig. 1, Table A1)."*

Why was the total time of interest not included in the PCA? There is no information provided on the Kaiser-Meyer-Olkin test for sampling adequacy or Bartlett's test of sphericity – were the data suitable for PCA? Why was only the first principal component (PC1) used in subsequent analyses, given that it explained only about 49% of the variance in the original variables, while PC2 had an eigenvalue greater than 1? Additionally, why was the number of flyovers associated with PC1 if its correlation was higher with PC2?

- Methods. *"We ran general linear models with the aggressiveness scores (PC1) as a continuous response variable, and one continuous variable (day within the season) plus two categorical variables (experimental group: naïve versus experienced males; year when the experiment was conducted) as predictors."*

Multiple trials of the same type of experiment conducted across different years represent random variations or levels of a random effect rather than fixed, controlled conditions (if there is no specific reason to treat the year as a fixed factor). Therefore, I would include the year in the model as a random factor to account for variability between years.

General comment: It is challenging to rule out the possibility that the naïve males were predominantly 2cy individuals (information on return rates would be useful here) and, for some reason, were paired later than experienced males. If this is the case, a lack of significant effect from the variable of season progress alone may not be very informative.

#### c) Chapter 3

- Methods. *"For Tawny Pipits, categories spanned from the local scale (birds within a 3.5 km radius of the focal individual) to broader scales including birds from the same field site but between 3.5 km and 10 km, and over 10 km away, birds from different field sites in the*

same country (excluding Czechia and Italy with only one site), and birds from different countries. Similarly, for Berthelot's Pipits, the categories ranged from local scale (within 3.5 km) to four more broader scales: same island (3.5 to 10 km, and >10 km away), different islands within the same archipelago, and different archipelagos (Canaries vs. Madeira)."

I understand that the paper focuses on macrogeographic variation in bird song. However, it would be fascinating to explore how similar the single-song repertoires or specific song features are among neighbouring males. This information would be highly relevant to the discussion on cultural transmission (which is thoroughly addressed in the dissertation), as learning processes generally occur on a local scale rather than across broader landscapes. This may be especially important for the Berthelot's Pipit, which is a resident species. Additionally, why was a 3.5 km radius selected?

- Discussion. "A narrow range of potential tutors seems plausible for Berthelot's Pipits since they are territorial year-round (Alström & Mild 2003) and maintain stable territories over time (Juan Carlos Illera, pers. comm.)."

Is this species truly territorial throughout the year, actively defending its territory even in the autumn?

## **6. Other observations on the content or form of the dissertation**

Minor comments on:

### a) Chapter 1

- Introduction. "Recognition might also take place at the individual level if birds show consistent individually distinctive patterns in their acoustic signals (e.g., Terry et al. 2005; Osiejuk et al. 2007; Petrusková et al. 2016). In this way, birds can discriminate between familiar and unfamiliar conspecifics in different social contexts (e.g., neighbour vs. stranger discrimination, parent-offspring recognition, kin recognition; Lambrechts and Dhondt 1995)."

However, distinguishing between familiar and unfamiliar individuals does not necessarily meet the criteria for individual vocal recognition. It may involve simply differentiating between two categories, as in classic neighbour-stranger discrimination, where it is unclear whether the bird recognizes a specific neighbour or merely identifies a familiar song.

- Introduction. "Most of the existing studies have focused on non-passerine species with simple vocalizations, such as Great Bitterns (Gilbert et al. 2002), ducks (Volodin et al. 2005), and owls (Galeotti and Sacchi 2001; Tripp and Otter 2006; Grava et al. 2008; Choi et al. 2019)."

It is possible that the studies focused on non-passerine species not due to their simple vocalizations, but because they are difficult to identify through traditional methods (e.g. colour ring combinations), for instance, due to their nocturnal activity.

- Introduction. "Nonetheless, there is still a lack of documentation on female songs for most songbirds, especially in temperate areas where studies on female songs are much less prevalent than in the tropics (Langmore 1998; Odom et al. 2014; Hall and Langmore 2017)."

The studies on female song of temperate zone bird species might be less prevalent as the female singing is just not so common as in the tropics.

- Discussion. *“As reported in a congeneric species, the Tree Pipit (Petrušková et al. 2016), IAM seems more reliable than ring observations for determining the density of singing individuals as well as estimating their return rates and within-season spatial territorial dynamics.”*

Even as a big fan of bioacoustics-based solutions, I believe that IAM does not necessarily have a clear advantage over using colour rings, as its effectiveness depends on the species. For instance, if an individual alters its song or repertoire within or between seasons, or if there is some song sharing among neighbouring individuals, IAM may not be as effective. I would just try avoiding generalizations.

#### b) Chapter 2

- Results. *“All 60 tested tawny pipit males reacted to the test stimulus, most of them promptly, within seconds of the initiation of the stimulus playback (mean value of latency to reaction was 9 s). Only 10 males (eight of them experienced) reacted later, with latency exceeding 15 s, but all responded within the first minute of the recording playback.”*

It would be more informative if the mean values were accompanied by measures of range.

- Results. *“A notable outlier was an experienced male that responded to the playback after 10 s, but flew away 30 s later to join the female, in whose close vicinity it spent the rest of the experimental period, presumably mate guarding; all other males expressed interest for at least 210 s.”*

Should not the male be excluded from the analysis since the trial did not meet the experimental criteria, which required the absence of stimuli other than the treatment stimulus? In fact, the presence of the female could have influenced nearly every aspect of its behaviour if the pair was at different breeding stage.

- Results. *“Figure A2. Paired box plots illustrating differences in song rate before and after playback experiments for (a) experienced and (b) naïve males tested in 2016.”*

To improve readability, panels a) and b) could be omitted, and the song rates of experienced and naïve males could be displayed side by side for clearer comparison.

#### c) Chapter 3

- Methods. *“The Tawny and Berthelot’s Pipits are two sister species of the motacillid genus *Anthus*, which comprises over 40 species distributed worldwide (Fitzpatrick et al. 2004).”*

This sentence partially reiterates content already covered in the introduction.

- Methods. *“(In cases when a male sung two distinct song types, we included each of them separately in the analyses if the recording quality was sufficient).”*

The brackets are unnecessary here.

- Results. *“The songs were short, usually around 0.4 to 0.5 s for Tawny Pipits and 0.3 to 0.4 s for Berthelot’s Pipits, and varied in complexity among birds.”*

Including some range measures would be helpful.

- Results. *“Fig. 3. UPGMA dendrogram of song similarity based on the dynamic time warping algorithm implemented in Luscinia software.”*

I understand the figure represents the analysis of Berthelot’s Pipit songs, but it would be helpful to mention the species in the figure caption for clarity.

I, hereby, declare that the reviewed PhD thesis by **Javier Oñate Casado** meets the criteria for doctoral theses and I recommend it for further stages of doctoral proceedings.

.....13.09.2024.....

Date



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Reviewer's signature