SYSTEMATICS OF THE SOUTHERN AFRICAN LARKS (ALAUDIDAE): SYRINGEAL AND VOCALISATION PERSPECTIVE

MASTER OF SCIENCE IN ZOOLOGY

A NTHANGENI

2021

SYSTEMATICS OF THE SOUTHERN AFRICAN LARKS (ALAUDIDAE): SYRINGEAL AND VOCALISATION PERSPECTIVE

by

ALUWANI NTHANGENI

RESEARCH DISSERTATION
Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN ZOOLOGY

in the

FACULTY OF SCIENCE AND AGRICULTURE
(School of Molecular and Life Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: PROF TG MANDIWANA-NEUDANI
CO-SUPERVISOR: PROF GD ENGELBRECHT
CO-SUPERVISOR: DR C KOPUCHIAN
(Centro de Ecología Aplicada del Litoral-CONICET, Argentina)

2021

Clade A - *Alaudid representative species*

Large-billed Lark *Galerida magnirostris*

Horned Lark *Eremophila alpestris*

Red-capped Lark *Calandrella cinerea*

Asian Short-toed Lark *Alaudala cheleensis*

Pink-billed Lark *Spizocorys conirostris*

Eurasian Skylark *Alauda arvensis*

(*Courtesy: Google Advanced Search - free to use or share*)

Clade B – *Mirafrid representative species*

Rufous-naped Lark *Mirafra africana*

Horsfield's Bush Lark *Mirafra javanica*

Cape Clapper Lark *Mirafra apiata*

Rudd’s Lark *Heteromirafra ruddi*

Karoo Lark *Calendulauda albescens*

Sabota Lark *Calendulauda sabota*

(*Courtesy: Google Advanced Search - free to use or share*)

Clade C - *Ammomanid representative species*

Cape Long-billed Lark *Certhilauda curvirostris*

Madagascan Lark *Eremopterix hova*

Grey-backed Sparrow-Lark *Eremopterix verticalis*

Spike-heeled Lark *Chersomanes albofasciata*

Desert Lark *Ammomanes deserti*

Greater Hoopoe-Lark *Alaemon alaudipes*

(*Courtesy: Google Advanced Search - free to use or share*)

DEDICATION

I DEDICATE THIS DISSERTATION TO MY MOM FOR ALWAYS ENCOURAGING ME TO REACH FOR MY LIMITS. SHE SHARES THE BELIEF WITH NIKOLA TESLA WHO SAID "LET THE FUTURE TELL THE TRUTH AND EVALUATE EACH ONE ACCORDING TO HIS WORK AND ACCOMPLISHMENTS. THE PRESENT IS THEIRS; THE FUTURE, FOR WHICH I HAVE REALLY WORKED, IS MINE". WITHOUT HER, THIS PROJECT WOULD HAVE NEVER BEGUN. MY LATE FATHER, THIS IS FOR YOU. TO MATHABO, WHOSE LOVE AND SUPPORT INSPIRED ME AND MADE THIS ENDEAVOUR EASY TO ENDURE.

DECLARATION

I hereby declare that this piece of work titled: **SYSTEMATICS OF THE SOUTHERN AFRICAN LARKS (ALAUDIDAE): SYRINGEAL AND VOCALISATION PERSPECTIVE** is my own work in design and in execution and that all the sources utilised or cited have been indicated and acknowledged as such in the reference and acknowledgement sections. This dissertation has not been previously submitted for the degree at this or any other university and I therefore present it for examination to the University of Limpopo for the degree of Master of Science in Zoology that is, MSc (Zoology).

Nthangeni A (Mr) 12 July 2021

……..... …….

…….

Signature

ACKNOWLEDGEMENTS

I wish to acknowledge and thank the people who contributed throughout the course of my study and institutions that provided the resources and support for making this study a success. Firstly, I feel indebted and also grateful to the following persons and institutions:

- my supervisors (Professor Tshifhiwa G Mandiwana-Neudani, University of Limpopo), Professor Derek G Engelbrecht, University of Limpopo) and Dr Cecilia Kopuchian (Centro de Ecología Aplicada del Litoral-CONICET, Argentina) for their immense professional guidance and the trust they had in me for the successful execution of this research project.
- Mr Dawie H de Swardt (National Museum, Bloemfontein) for his assistance with the collection of bird specimens. He taught me to prepare bird skins and also provided some sound recordings.
- Professor Per J Alström (Uppsala University, Sweden) is thanked for additional vocalisations of some lark species.
- I would like to give thanks to Ms Elna Cowley and Professor Chantelle Baker (Electron Microscope Unit, Sefako Makgatho Health Sciences University) allowing me access to the laboratory and taking me through the histological processing of the specimens.
- the additional sound recordings of larks were sourced from online databases including Cornell University Macaulay Library of Natural Sounds (MLNS), Xeno-canto (http://www.xeno-canto.org/), Avian Vocalizations Center (AvoCet, http://avocet.integrativebiology.natsci.msu.edu) and Desmond Allen for the Horsfield's Lark uploaded in the Internet Bird Collection (IBC).
- my mother for understanding that education is the key to a better future and my late father who always taught to never give up on my dreams. His role can never be measured.
- my brother and sister for always pushing me to higher limits by continuously giving me a big cheer throughout the course the study.
- lastly, my girlfriend Mathabo whose love and support gave me courage to carry on with my studies until completion.

PREFACE

Part of the results gained in this study were presented at national and local conferences as follows:

1. Nthangeni A, Engelbrecht GD, Kopuchian C, Mandiwana-Neudani TG. Assessing the utility of vocalisations of larks (Alaudidae) in a systematic perspective. African Bioacoustics Community (ABC) Conference. University of Cape Town, 2–7 December 2018. Paper.

2. Nthangeni A, Engelbrecht GD, Kopuchian C, Mandiwana-Neudani TG. Systematics of the southern African larks (Alaudidae): vocalisation and syringeal perspective. 13th Southern African Society for Systematic Biology (SASSB), National Botanical Gardens, Pretoria, 2–4 July 2017. Poster and Short talk.

3. Nthangeni A, Engelbrecht GD, Kopuchian C, Mandiwana-Neudani TG. Systematics of the southern African larks (Alaudidae): vocalisation and syringeal perspective. The Faculty of Science and Agriculture's Postgraduate Research Day. Bolivia Lodge, Polokwane, 19–20 October 2017. Paper (1st Prize).

ABSTRACT

The larks (Passeriformes, Passeri, Alaudidae) are small to medium-sized (10-23 cm) birds that are primarily terrestrial and cryptically plumaged hence they are difficult to encounter and recognise. The current taxonomic circumscription places these birds in a group that is comprised of 21 genera and 98 species, with all the genera occurring in Africa, 13 in Eurasia, and a single genus occurs in Australia and the Americas. Up until Alström *et al.* (2013), morphologically, the lark family was distinguished by having two unique and primitive features: i) the tarsus morphology (latiplantar and scutellate) consisting of the flat posterior surface covered with prominent scales, instead of being narrow and smooth as in other families, and ii) the syrinx (voice-generating organ). Despite that the structure of the syrinx of larks has been studied, literature reveals confusion pertaining to either the presence or absence of the pessulus, its level of development and size. To date, the work in Alström *et al.* (2013) remains the most comprehensive multi-locus phylogeny of the larks in which three strongly supported major clades (clade A – hereafter the Alaudid, clade B – the Mirafrid, clade C – the Ammomanid) emerged though with some uncertainty in some parts of the tree. In this study, the aim was to investigate the utility of syringeal and vocal characters in classifying the species of larks, finding out how syringeal and vocal characters evolved and identifying characters that define clades. The gross morphology and histology of the syringes and song strophes of larks and their putative outgroups were studied.

Gross morphologically and histologically, the larks were found to possess a typical syrinx classified as a '*syrinx tracheo-bronchialis*' and pessulus was observed in larks and the outgroups studied. There were differences observed in the syringeal gross morphological structure across all the three major clades (A, B and C). This is with regard to the presence or absence of the divided or double bronchial rings variably observed in clade A, B and C. In clade B and C, the ossification is variably restricted to the centre of bronchial rings forming a serial pattern while in clade A, bronchial rings are variably almost fully ossified without forming any serial pattern. The prominent oblique muscle-like structure runs ventrally and it was only observed in clade C in *Chersomanes albofasciata.* On the other hand, the syringeal histology revealed differences in the shape of the pessulus (blunt, pointy or sharp), the pessulus position relative to bronchial rings 1, 2 and 3 (B1, B2 and B3 respectively), length of the internal

tympaniform membranes and connective tissue along the internal tympaniform membrane. The position of the pessulus was variably found to align with B2, to be below B2 and to be positioned beyond B2. One-way Anova clearly showed that among the three clades (A, B and C) identified in Alström *et al.* (2013), a statistically highly significant difference ($P < 0.01$) was found between the song strophes of species in clade C and A. The species in clade A generally give song strophes defined by high maximum frequency, high peak frequency and broad bandwidth frequency. The species in clade B have a similar trend with those in clade A, possibly explaining the overlap between these clades and the statistically significantly difference between clade A and C. These findings may be in support of the phylogenetic findings in Alström *et al.* (2013) and this study wherein clade A and B shared a sister relationship while clade C was placed basally. Clade C, on the other hand, comprises song strophes that are defined by low maximum frequency, lower peak frequency and narrow bandwidth frequency and this clade differed significantly from clade A. Despite that not all of the species could be correctly classified to their respective clades based on the Discriminant Function Analysis' partition plot, the largest number of correct classifications were for clade A (70%). In addition, the distinction among the clades was also observed in either the presence or the absence of wing clappings in the song strophes, either being detached from or attached to the song strophes. Clade B is the only one which was marked by the presence of wing clappings particularly, genus *Mirafra*, although they are reported in *Chersophilus duponti* which belongs to clade A but not included in this study. With regard to the vocal phylogeny, the topology was highly unresolved, and no relationships could be inferred. The tracing of the evolution of characters of eight vocal and five syringeal characters revealed that among the 13 characters for which the ancestral state reconstructions were performed, 12 are polymorphic that is, they underwent multiple state changes ranging from four to 18. Most character states were found to plesiomorphous and mainly leading to clades of which their ancestral nodes were defined largely by autapormorphic and symplesiomorphic states. These do not assist in explaining how the various characters evolved. In conclusion, the findings have shed some light concerning the general syringeal morphology and histological structures of larks, revealed that lark songs are not suitable for reconstructing the phylogeny, shed light on the evolution of the selected vocal and syringeal characters as well as identifying characters that define the three major clades of larks (the Alaudid, Mirafrid and the Ammomanid).

TABLE OF CONTENTS

DEDICATION ..... **i**

DECLARATION ..... **ii**

ACKNOWLEDGEMENTS ..... **iii**

ABSTRACT ..... **v**

TABLE OF CONTENTS ..... **vii**

LIST OF FIGURES, TABLES AND APPENDICES ..... **x**

CHAPTER 1 ..... **1**

General Introduction **1**

1.1 Characteristics, distribution and diversity of larks 1

1.2 The state of taxonomy and phylogeny of larks 2

1.3 The latest phylogeny of larks as the departure point 4

1.4 What is systematics? 5

1.4.1 The common documented approaches in systematic studies 6

1.4.2 Tracing the evolution of characters 9

1.5 Sparse versus dense sampling in the systematic study 9

1.6 Purpose of the study 10

1.6.1 Study aim 10

1.6.2 Study objectives 10

1.6.3 Study research questions 11

CHAPTER 2 ..... **18**

Gross morphological and histological description of the syrinx of larks (Passeriformes, Alaudidae) **18**

2.1 Introduction 18

2.1.1 What is a syrinx? 18

2.1.2 The history of syrinx morphology 21

2.1.3 Errors noted in previous studies in the anatomy of the syrinx 24

2.1.4 What do we know about the syrinx of larks? 25
2.1.5 Aim 28
2.1.6 Objectives 28
2.2 Materials and methods 28
2.2.1 Sampling of syringes 28
2.2.2 Gross morphological and histological examination of syringes 29
2.3 Results 31
2.3.1 Syringeal gross morphology 31
2.3.2 Syringeal histology 35
2.4 Discussion and Conclusions 37
CHAPTER 3 107
The characterisation of larks (Passeriformes, Alaudidae) based on vocalisations 107
3.1 Background information 107
3.1.1 Vocalisations and classification of taxa 108
3.1.1.2 Data types used to characterise larks 113
3.1.2 Aim 114
3.1.3 Objectives 114
3.2 Materials and methods 115
3.2.1 Data collection 115
3.2.2 Data analyses 116
3.3 Results 119
3.3.1 Univariate findings – one-way ANOVA 119
3.3.2 Multivariate findings – Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) 119
3.3.3 What do song strophes tell about the grouping of species within each clade and across genera? 121
3.3.4 Description of song strophes 124
3.4 Discussion and Conclusions 136
CHAPTER 4 214

The evolution of vocal and syringeal characters traced on a phylogeny using a parsimony-based method 214

4.1 Background information 214

4.1.1 Ancestral state reconstruction and phylogenies 214

4.1.2 Study taxa 215

4.1.3 Aim 217

4.1.4 Objectives 217

4.2 Materials and methods 218

4.2.1 Taxon and character sampling 218

4.2.2 Analysis 219

4.3 Results 220

4.3.1 Molecular and vocal phylogenetic outcomes 220

4.3.2 Ancestral state reconstructions 221

4.4 Discussion and Conclusion 224

CHAPTER 5 250

Synthesis and future prospects 250

5.1 Highlights on key findings 250

5.2 Future prospects 252

REFERENCES 253

LIST OF FIGURES, TABLES AND APPENDICES

Figure 1.1. Map showing the global distribution of larks (Alaudidae). Sourced from de Juana *et al.* (2020). 12

Figure 1.2. Phylogenetic tree of the lark family, Alaudidae as sourced from Alström *et al.* (2013). Different colours of names indicate genera as defined by Peters (1960) based on morphology; monotypic genera are shown in black. Revised names compared to Gill and Donsker (2012) are indicated by *. 13

Figure 1.3. Phylogenetic tree of the lark family, Alaudidae as sourced from Alström *et al.* (2013). Majority rule (50%) consensus tree of Alaudidae based on concatenated nuclear ODC, myoglobin and RAG1 + 2 and mitochondrial cytochrome *b* (*cytb*) and 16S sequences, inferred by Bayesian inference, analysed in five partitions. 14

Figure 2.1. Examples of variation in syringeal anatomy. The tracheal parrot syrinx has two syringeal muscles and a pair of lateral tympaniform membranes. The bronchial syrinx of the oilbird has one pair of syringeal muscles and a pair of medial and lateral tympaniform membranes in each bronchus. Songbirds have several pairs of syringeal muscles in their tracheobronchial syrinx. Tr, trachea; ST, sternotrachealis muscle; SY SUP, superficial syringeal muscle; SY PROF, deep syringeal muscle; SY VALV, pneumatic valve; LTM, lateral tympaniform membrane; MTM, medial tympaniform membrane; BCI, first bronchial cartilage; SY, syringeal muscle; ML, medial labium; LL, lateral labium; SYR, muscles of syrinx. (Oilbird modified after Suthers and Hector 1985; parrot and canary modified after King 1989)…….. 46

Figure 2.2. Example of a ventral view of the syrinx of a Red-capped Lark *Calandrella cinerea* illustrating the *syrinx tracheo-bronchialis* found in larks. The terminology was adopted from Düring *et al.* (2013). 47

Figure 2.3. Example of a ventral view of the syrinx of a Pink-billed Lark *Spizocorys conirostris* showing different muscles and cartilages found in larks. Terminology was adopted by Düring *et al.* (2013). 48

Figure 2.4. The three major differences in the gross morphological structure of the selected syringes of larks across all the clades: a. the degree of bronchial ring ossification (Figs. 2.4a and b have restricted ossification while 2.4c and d are almost fully ossified), b. bronchial ring ossification pattern (serial pattern in Figs. 2.4a and b and non-serial pattern in Figs. 2.4c and d) and c. bronchial ring completeness (almost joined/closed C-bronchial ring – Figs. 2.4c or open C-bronchial rings – Figs. 2.4a, b and d). 49

Figure 2.5. The hematoxylin and eosin stained histological section of a typical lark (a. Large-billed Lark *Galerida magnirostris*) showing the syringeal tissue structures used to compare different species and the outgroups (b. Rattling Cisticola *Cisticola chiniana*) used in the study..... 51

Figure 3.1. Examples of spectrograms of song strophes of larks ranging from simple (a. Agulhas Long-billed lark *Certhilauda brevirostris*), less complex (b. Dune lark *Calendulauda erythrochlamys*) and complex (c. Sabota lark *Calendulauda sabota*). E – Element, E1 – Element number 1, N_{ele} – Number of elements, F_{min} – minimum frequency, F_{max} – maximum frequency, F_{band} – bandwidth frequency. 157

Figure 3.2. Scatterplot showing a greater overlap between clade (group) A and clade B than between both A and B and clade C..... 159

Figure 3.3. Scree Plot showing the Eigenvalues and Components extracted relative to the variance of the three significantly different variables (F_{max}, F_{peak} and F_{band}) used in Principal Component Analysis at clade level. 159

Figure 3.4. Biplot of Principal Component (PC) 1 and PC2 scores from Principal Component Analysis (PCA) from the three significantly different variables (F_{max}, F_{peak} and F_{band}) at clade level. In the abbreviation C.ch, C stands for clade C, chu represents the specific epithet '*chuana*'. Refer to Appendix 3.1 for all species names. 160

Figure 3.5. Partition plot from Discriminant Function Analysis (DFA) based on significantly different variables showing the separation of the three major clades based on PC1 and PC2. Alphabets in black colour show the correct grouping of each of the clades (A, B, C) and red alphabets represent clades that are incorrectly grouped. The

Pink shade is clade C cluster, blue shade is clade A cluster and white shade is clade B cluster. Refer to Appendix 3.1 for species names. 161

Figure 3.6. Scree Plot showing the Eigenvalues pertaining to variance for each component extracted. Four variables (F_{max}, F_{peak}, N_{ele} and S_{dur}) were used in Principal Component Analysis (PCA) for clade A. 163

Figure 3.7. Biplot of principal component 1 (PC1) and PC2 scores from principal component analysis (PCA) of all four variables (F_{max}, F_{peak}, N_{ele} and S_{dur}) at clade A. In the abbreviation C.acu, C stands for genus *Calandrella*, acu represents the specific epithet '*acutirostris*'. Refer to Appendix 3.1 for species names. 163

Figure 3.8. Partition plot from Discriminant Function Analysis (DFA) at clade A based on PC1 and PC2. Refer to Appendix 3.1 for species names. A = *Galerida*, B = *Calandrella*, C = *Eremophila*, D = *Alauda*, E = *Chersophilus*, F = *Alaudala*, G = *Melanocorypha*, H = *Spizocorys*, I = *Lullula*. 164

Figure 3.9. Scree Plot showing the Eigenvalues pertaining to variance for each component extracted. Four variables (F_{max}, F_{min}, F_{peak} and S_{dur}) used in Principal Component Analysis (PCA) for clade B. 166

Figure 3.10. Biplot of principal component 1 (PC1) and PC2 scores from Principal Component Analysis (PCA) from four variables (F_{max}, F_{min}, F_{peak} and S_{dur}) at clade B. In the abbreviation Ca.afri, Ca stands for Genus *Calendulauda*, afri represents the specific epithet '*africanoides*'. Refer to Appendix 3.1 for species names. 166

Figure 3.11. Partition plot from Discriminant Function Analysis (DFA) at clade B based on PC1 and PC2. Refer to Appendix 3.1 for the species name. J = *Mirafra* Southern, K = *Mirafra* Northern, L = *Heteromirafra*, M = *Calendulauda*. 167

Figure 3.12. Scree Plot showing the Eigenvalues pertaining to variance for each component extracted. Four variables (F_{max}, N_{ele}, F_{peak} and S_{dur}) used in Principal Component Analysis (PCA) for clade C. 169

Figure 3.13. Biplot of principal component 1 (PC1) and PC2 scores from Principal Component Analysis (PCA) from four variables (F_{max}, N_{ele}, F_{peak} and S_{dur}) at clade C.

In the abbreviation Er.nig, Er stands for genus *Eremopterix*, nig represents the specific epithet '*nigriceps*'. Refer to Appendix 3.1 for species names. 169

Figure 3.14. Partition plot from Discriminant Function Analysis (DFA) at clade C based on PC1 and PC2. Refer to Appendix 3.1 for species names. N = *Certhilauda*, O = *Eremopterix*, P = *Ammomanes*, Q = *Ramphocorys*, R = *Ammomanopsis*, S = *Chersomanes*, T = *Alaemon*..... 170

Figure 4.1. A strict consensus pruned parsimony phylogeny of larks (Alaudidae) generated from combined mitochondrial and nuclear DNA sequence characters. Numbers shown above branches are bootstrap values (only ≥ 50%). 235

Figure 4.2. A strict consensus parsimony phylogeny of larks (Alaudidae) generated from vocal characters. 236

Figure 4.3. Ancestral character state reconstruction based on parsimony optimisation of wing clapping traced on a combined mitochondrial and nuclear DNA phylogeny of larks..... 237

Figure 4.4. Ancestral character state reconstruction based on parsimony optimisation of wing clapping incorporation in song traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 238

Figure 4.5. Ancestral character state reconstruction based on parsimony optimisation of grouped element-ending traced on a combined mitochondrial and nuclear DNA phylogeny of larks…..... 239

Figure 4.6. Ancestral character state reconstruction based on parsimony optimisation of grouped element-ending structure traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 240

Figure 4.7. Ancestral character state reconstruction based on parsimony optimisation of strophe type traced on a combined mitochondrial and nuclear DNA phylogeny of larks..... 241

Figure 4.8. Ancestral character state reconstruction based on parsimony optimisation of strophe length traced on a combined mitochondrial and nuclear DNA phylogeny of larks..... 242

Figure 4.9. Ancestral character state reconstruction based on parsimony optimisation of strophe pitch traced on a combined mitochondrial and nuclear DNA phylogeny of larks..... 243

Figure 4.10. Ancestral character state reconstruction based on parsimony optimisation of mimicry traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 244

Figure 4.11. Ancestral character state reconstruction based on parsimony optimisation of divided bronchial rings traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 245

Figure 4.12. Ancestral character state reconstruction based on parsimony optimisation of the oblique structure on ventral side traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 246

Figure 4.13. Ancestral character state reconstruction based on parsimony optimisation of degree of bronchial ring ossification traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 247

Figure 4.14. Ancestral character state reconstruction based on parsimony optimisation of bronchial ring ossification pattern traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 248

Figure 4.15. Ancestral character state reconstruction based on parsimony optimisation of bronchial ring completeness traced on a combined mitochondrial and nuclear DNA phylogeny of larks. 249

Table 2.1. List of the Lark and Outgroup species included in the gross morphological analysis of the syringes examined..... 41

Table 2.2. List of the Lark and Outgroup species used for histological analysis of the syringes examined. 45

Table 3.1. List of song recordings from which song strophes were selected. MLNS = Cornell University's Macaulay Library of Natural Sounds, AvoCet = Avian Vocalization Centre, PA = Per Alström, DA = Desmond Allen, XC = Xeno-canto. Bolded numbers indicate song recordings from which reference song strophes were extracted. 142

Table 3.2. List of lark species, spectrogram and aural characteristics generated from song strophes of each species. 147

Table 3.3. The mean and standard deviation for each variable across all clades, including the *F*-statistic and *p*-value from the ANOVA at clade level. An asterisk indicates significance at the *P* = 0.05 level. A hereafter represents the Alaudid clade, B – Mirafrid clade and C – Ammomanid clade. 150

Table 3.4. The variables extracted by Principal Component Analysis (PCA) with the first two PC scores for the clade. Eigenvalues and percentage variance each component explain in the data are shown. Loadings (|0.5|) are indicated by an asterisk. 151

Table 3.5. *Post-hoc* Tukey's Honestly Significant Difference (HSD) test for the two principal component (PC) scores significantly differ at clade level. 152

Table 3.6. Discriminant Function Analysis (DFA) prediction of species membership in the three clades (A, B and C). Summation of species is vertical. Asterisk (*) indicates the correct classification. 152

Table 3.7. The *F*-statistic and *p*-values from Analysis of Variance (ANOVA) for all the variable used within each clade. An asterisk indicates significance at 0.05 level. .. 153

Table 3.8. *Post-hoc* Tukey's HSD test for ANOVA showing significant differences between genera across all the three clades (A, B and C). 154

Table 3.9. The variables extracted by Principal Component Analysis (PCA) with the first two principal components (PC) scores for clade A, clade B and clade C, respectively. Eigenvalues and percentage variance each component explain in the data within each clade are shown. Loadings (|0.5|) are indicated by Asterisk. 155

Table 3.10. *Post-hoc* Tukey's Honestly Significant Difference (HSD) test for Principal Component (PC) 1 and PC2 showing significant differences between genera across clade A, clade B and clade C. 156

Table 4.1. List of ingroup and outgroup species taxa analysed in phylogenetic reconstructions. GenBank accession numbers are provided. 228

Table 4.2. A character matrix showing the scores for the character states as used in the reconstruction of vocal phylogeny (character states 1 – 8). Characters 9 – 13 represent the syringeal characters used in the reconstruction of ancestral character states only……231

Table 4.3. The vocal and syringeal characters and the scores of their respective character states as used in the reconstructions of character states. 234

Appendix 1.1. List of species of larks of the world. English and specific names are as proposed in the IOC World Bird List (Gill and Donsker 2020). The clades follow the circumscription in Alström *et al.* (2013). '*' denotes the species that were not included in Alström *et al.* (2013). 15

Appendix 2.1. Gross morphology plates for clade A. For labelling, e.g. Appendix 2.A.1 a-c), 2 refers to appendix number; **A** – clade; 1 – species *Calandrella cinerea*; number 62a – series of pictures of the same syrinx of an individual bird; 63b – series of pictures of the same syrinx of another individual. NB. Red colour – shows ossified cartilage, blue – unossified cartilage, light brownish colour – muscle. 52

Appendix 2.2. Gross morphology plates for clade B. 63
Appendix 2.3. Gross morphology plates for clade C. 84
Appendix 2.4. Gross morphology plates for outgroups. 96
Appendix 2.5. Histological sections of syringes of different species of larks and outgroups species. For labelling, e.g. (Appendix 2.5.1 127a-c), 2 refers to appendix number; 5 – histological sections; 1 – a particular species – Galerida magnirostris; 127 – sample number for Galerida magnirostris syrinx, a – c a series of tissue sections. NB. All sections with number 127 come from a syrinx of an individual bird and 113 is another individual bird but the same species Galerida magnirostris with a-g showing series of sections..... 101

Appendix 3.1. List of species used in Chapter 3 including the codes used in the Biplot and partition plots. 171
Appendix 3.2. Multiple spectrograms of song strophes generated from different individuals representing different species. **ML** - Macaulay Library of Natural Sounds, **XC** - Xeno-canto, **PA** - Per Alström, **DS** - Dawie de Swardt, **DA** - Desmond Allen, **AV** - AVoCet). For figure numbering, e.g. (**3.2.1 a-f**), **3** denotes chapter number, **2** denotes appendix number, **1** denotes genus, **a-f** denote species. 173

CHAPTER 1

General Introduction

1.1 Characteristics, distribution and diversity of larks

The larks are a group of small to medium-sized (10-23 cm) birds generally perceived to be 'greyish-brown' coloured birds that are difficult to encounter and identify. This is due to their mostly terrestrial habitats and cryptic colouration correlating to the colour of the soil they inhabit (Meinertzhagen 1951; Hockey *et al.* 2005; Guillaumet *et al.* 2008). Most species show no sexual dimorphism, although males average larger than females (Cramp 1988). They have long, straight and narrow hind claw related to terrestrial passerines such as pipits (*Anthus*) and long claws (*Macronyx*) of the family Motacilidae (de Juana *et al.* 2020). Larks can be found in some of the most hostile habitats on Earth but can accomplish their greatest diversity in such environments (Dean and Hockey 1989). Nonetheless, they experienced adaptive radiation matched by only a few other avian families and their superficial similarity controverts enormous variation between species, ecology, distribution, behaviour, social organisation and population (Donald 2004).

The larks are primarily terrestrial and cryptically plumaged birds and belong to the order Passeriformes, suborder Passeri, which contains Oscines or songbirds and belong to family Alaudidae. All genera occur in Africa, followed by Eurasia having 13 genera, and only a single genus occurs in Australia and the Americas (de Juana *et al.* 2020; Gill and Donsker 2020) (Fig. 1.1). The family comprises 21 genera which host 98 currently recognised species (Appendix 1.1) depending on whether closely related groups of species are considered a single species (de Juana *et al.* 2020). Seventy-eight lark species occur in Africa, with 60 endemic species found in the sub-Saharan African region. In Eurasia 36 species occur of which 17 are endemic, and the New World has only one species, the Horned Lark *Eremophila alpestris*. The other species occurring outside Africa-Eurasia are the Australian Bush Lark *Mirafra javanica* which ranges from south-east Asia to Australia.

Larks are also present on the islands that are far away from the continents or that were part of continents such as the Balearic Islands, Cape Verde, Archipelago and Faroe Islands (de Juana *et al.* 2004). The current distribution and diversity of the family confirm that it is primarily an African, and secondarily a Eurasian family (Fig. 1.1).

Barnes (2007) described the distribution of larks as skewed and having two "hot spots" of diversity, matching to the arid zones of the north-east (Kenya, Ethiopia and Somalia) and south-west of Africa (South Africa, Namibia and Botswana). There are 37 species, of which 62% are endemic to the north-east arid zone, while the south-west arid zone holds no fewer than 33 species, of which 85% are endemic or near endemic to this region (Dean and Hockey 1989; Barnes 2007). The combined total number of species in these two regions adds up to 65 species, approximately 83% of the African total and 68% of the larks in the world. According to White (1961) and Moreau (1966), these two arid zones may have previously been linked by passage through present-day Kenya, Tanzania, Zambia, and Zimbabwe before they were geographically isolated.

1.2 The state of taxonomy and phylogeny of larks

Morphologically, the lark family is distinguished by two unique and primitive features, i) the tarsus morphology (latiplantar and scutellate) comprising the posterior surface flat and covered with prominent scales, instead of being narrow and smooth as in other families, and ii) the syrinx (voice-generating organ) which is generally said to be either primitive (de Juana *et al.* 2020) or simple (Suárez *et al.* 2009) and lacking a bony central structure called a pessulus (Mayr and Amadon 1951; Ames 1971; King and McLelland 1984; Dean and Williams 2004). However, it should be noted that Ames in his 1971 classical paper, cited the findings in MacGillivray (1839) where a pessulus was found to be lacking in larks. Surprisingly, MacGillivray (1839) does not mention anything about a pessulus in any of the species he studied. According to Verheyen (1958), larks have a rudimentary pessulus, Suárez *et al.* (2009) and de Juana *et al.* (2020) based their information based on Verheyen (1958). The syringeal musculature of larks comprises only five pairs of muscles (Ames 1971). In his study of the morphology of the syrinx in Passerine birds, Ames (1971) studied the family Alaudidae but focusing on only a few genera and a few species representing each of these genera. Only seven genera and nine species were

included in Ames' study: *Alauda arvensis, Calandrella cinerea*, *Calandrella rufescens* (currently *Alaudala rufescens*), *Eremophila alpestris*, *Galerida cristata*, *Galerida modesta, Melanocorypha yeltoniensis, Mirafra angolensis* and *Mirafra sabota*. He stressed that the role of the pessulus in vocalisation is unknown as the lack of this syringeal feature does not affect singing in larks.

According to Dean *et al.* (1992) and de Juana *et al.* (2020), songbirds generally have between six and eight pairs of syringeal muscles and a well-developed pessulus (ossified). These structural features and recent molecular studies indicate that the *Alaudidae* is an ancient and highly distinct family of Oscine passerines with no near relatives.

The relationship between larks and other taxa has always been subjected to systematic scrutiny. The family was placed at the beginning of the Oscine passerine radiation based on the argument that the pessulus and tarsal features are primitive, not derived (de Juana *et al.* 2020). Since the outermost primary is reduced or vestigial, the family was placed among the nine-primaried songbirds (Keith *et al.* 1992; Donald 2004). Using DNA-DNA hybridisation, Sibley and Ahlquist (1990) and Sibley and Monroe (1990) placed the family in the superfamily Passeroidea. However, using conserved nuclear genes, Barker *et al.* (2002) showed them to be part of the superfamily Sylvioidea, and together with monotypic genus *Panurus* (Panuridae) as a sister to the rest of Sylvioidea (Fregin *et al.* 2012).

The phylogenetic assessment to assign genera has largely been based on morphology (bill structure and plumage) but they are undependable as the number of genera and species have been unstable over the years (de Juana *et al.* 2020; Alström *et al.* 2013), and Donald *et al.* (2017) showed that plumage features are less than adequate as a taxonomic feature of the larks. Approximately 20-23 genera representing Alaudidae have been variously presented by Donald (2004) and Alström *et al.* (2013). Therefore, the number of lark species has generally been undervalued as molecular and vocal data suggest there is considerable hidden diversity in larks, which has resulted in a taxonomic fluctuation in some taxa (Alström 1998; Ryan *et al.* 1998; Ryan and Bloomer 1999; Guillaumet *et al.* 2005; Guillaumet *et al.* 2008; Alström *et al.* 2013).

1.3 The latest phylogeny of larks as the departure point

Alström *et al.* (2013) produced what remains the most comprehensive multi-locus phylogeny of the lark species from mitochondrial and nuclear markers (Fig. 1.2). In their findings, they divided the larks into three (3) major clades (Clade A – the Alaudid, Clade B - Mirafrid and Clade C - Ammomanid) which were generally strongly affirmed by their data (Fig. 1.3). Clade A comprises the genera *Alaudala, Eremalauda, Chersophilus, Melanocorypha, Calandrella, Eremophila, Galerida, Alauda, Spizocorys* and *Lullula.* Sub-clade A1 contains *Calandrella*, *Melanocorypha, Eremophila* and two monotypic genera *Eremalauda* and *Chersophilus.* Previously, *Eremalauda* had frequently been placed close to *Ammomanes cintura* in Sub-clade C1 (Meinertzhagen 1951; Peters 1960; Wolters 1979; Pätzold 2003) [subgenus *Eremalauda*]), which is disproved by Alström *et al.* (013). Similarly, *Chersophilus* was also placed in *Certhilauda* along with *Alaemon* and *Chersomanes* based on bill structure and behaviour (Meinertzhagen 1951), but the data presented in Alström *et al.* (2013) strongly rejected the placement.

Within Sub-clade A2, a close association of *Galerida, Alauda* and *Melanocorypha leucoptera* was supported by the data. *Melanocorypha leucoptera* was further supported by a closer similarity to *Alauda* than to other *Melanocorypha* species or *Galerida* in morphology, vocalisation, behaviour and ecology (de Juana *et al.* 2020). As such, the species was renamed to *Alauda leucoptera* according to taxonomic priority principle. *Galerida magnirostris* and *G. modesta* have previously been placed in the monotypic genera *Calendula* (Wolters 1979; Pätzold 2003) and *Heliocorys* (Wolters 1979), respectively. The relationship between *Spizocorys* and *Lullula* was well-supported by data. Previously, a monotypic genus *Pseudalaemon* which contains Short-tailed Lark was found to be well-contained within the *Spizocorys* complex and the species was renamed *Spizocorys fremantlii* (Peters 1960; Wolters 1979; Dean *et al.* 1992; Dickinson 2003; Pätzold 2003; de Juana *et al.* 2020).

Clade B contains the genera *Mirafra, Heteromirafra* and *Calendulauda.* The relationship between *Mirafra* and *Heteromirafra* was well-supported by data and has formerly been hinted at in Dean *et al.* (1992). The relationship between *Calendulauda* and *Mirafra/Heteromirafra* was also supported. The five Asian species in sub-clade B1

are all morphologically similar but the relationship between them was mostly unsupported in Alström *et al.* (2013). This sub-clade consists of a mixture between African and Asian/Australian taxa. Sub-clade B2 consists of *Calendulauda* separated into two sub-clades that were well-supported by data.

Clade C comprises *Eremopterix, Ammomanes, Ramphocoris, Pinarocorys, Certhilauda, Ammomanopsis, Chersomanes* and *Alaemon.* In sub-clade C1, the genus *Eremopterix* was well-supported by data, but the relationship between some species was not well-resolved. The placement of *Eremopterix australis* and *Eremopterix hova* was highly uncertain. The relationships between *Ammomanes*, *Ramphocoris* and *Pinarocorys* were all supported by data, but varying support using different concatenated data was revealed between *Ammomanes* and *Ramphocoris*. Sub-clade C2 contains a trichotomy of lineages: all five *Certhilauda* species, two species of *Chersomanes* and *Ammomanopsis grayi*. Among the *Certhilauda* species, *Certhilauda chuana* was previously treated as *Mirafra* (Pätzold 2003), but the other species were treated as conspecific and this species is placed at the base in *Certhilauda* group. *Chersomanes* has been treated as *Certhilauda* and *Ammomanopsis grayi* has been placed in *Ammomanes* (Peters 1960; Dean *et al.* 1992; Dickinson 2003; Pätzold 2003). All the genera in sub-clade C1 were rooted in *Alaemon alaudipes* and it would be riveting to reveal whether *Alaemon hamertoni* is part of this clade or not.

1.4 What is systematics?

Systematics is the science of classifying organisms based on the similarities and differences of characters or features used by researchers in attempting to have classification systems reflecting their phylogeny (Schwartz 2011). A branch of systematics, ‘phylogenetics’, is the study and/or rebuilding of the evolutionary relationships between studied taxa, resulting in trees that signify the framework and hypothesis within which to study the ecology and evolution of organisms and traits that they exhibit (Fleagle 2013; Bjarnason *et al.* 2015). Accurate phylogenetic analysis needs shared similarity in taxa to be transmissible from a typical root (common ancestor), homology, instead of through merging (convergent) or parallel evolution, homoplasy (Hall 2007). A wide range of data types has become available in studies of systematics.

Evidence to study systematics, particularly avian systematics, can be sourced for example, from characters ranging from morphology, behaviour, anatomy, and nucleic acids sequences, but the use of nucleic acid sequences in systematics continues to be prominent (Felsenstein 1984; Cracraft *et al.* 2004; Edwards *et al.* 2005; Zou and Zhang 2016).

1.4.1 The common documented approaches in systematic studies

1.4.1.1 Morphology

The use of morphological characters in systematics continues even today (Bjarnason *et al.* 2015). Without disregarding the indisputable advantages of molecular characters, it is very important that we continue collecting morphological data for phylogenetic analysis and improve methods for morphological-based phylogenies (Wiens 2004). Several studies have shown that even though the phylogenies reconstructed from molecular data may present incontestable advantages, morphology-based phylogenies are important to validate the molecular results (Hillis and Wiens 2000; Karanovic *et al.* 2015).

Systematists have utilised characters from morphological features as the foundation of morphometry (Bookstein *et al.* 1985), ontogeny (Iwaniuk *et al.* 2006), and biogeography (Wiley 1988), and also for the study of patterns of speciation, and co-evolutionary interface (Alves *et al.* 2001) and conservation (Thompson and Newmaster 2014). Just like any set of characters, the main disadvantage is that morphological characters are not as numerous as molecular characters. Bocek and Bocak (2017) found that the intrageneric variability of most phenotypic traits of net-winged beetles and the limited number of characters supporting deep relationships in morphology does not provide enough support for a robust phylogeny. In a study of the American primates (platyrrhines), Bjarnason *et al.* (2015) showed that the cranial data had phylogenetic signals that closely reflected the molecular phylogeny. Furthermore, it should be highlighted that without the use, for example, of morphological and behavioural data to support these molecular phylogenies, these studies could be of limited benefit especially for scientists who manage populations of species.

1.4.1.2 Molecules

The nucleic acid sequences of different organisms are continuously generated into large datasets (Sayers *et al.* 2009) but some findings remain uncertain even when several markers from different genomes are used (Alström *et al.* 2013). Barker *et al.* (2002) presented the hypothesis of relationships of Oscine birds based on nucleotide variation at the nuclear RAG-1 and *c-mos* gene from 69 passerine taxa and reached a conclusion that the families Alaudidae, Irenidae and Melanocharitidae yielded strong evidence of misplacement in the hybridisation results. On the other hand, the mitochondrial variation of an endangered cyprinid fish *Anaecypris hispanica* endemic to Guadiana river basin in the Iberian Peninsula inferred distinctive Evolutionary Significant Units (ESUs) and within one of the ESUs, four Management Units (MUs) were considered (Alves *et al.* 2001). In a study to infer the phylogenetic position of the Wallcreeper *Tichodroma muraria*, two mitochondrial and five nuclear loci from *Tichodroma, Sitta, Certhia* and *Salpornis* were analysed and a sister relationship between *Tichodroma* and *Sitta* was strongly supported as well as between *Certhia* and *Salpornis* (Zhao *et al.* 2016).

Some of the advantages of using nucleic acid sequences in systematics is that they are cheaper than morphological data in relative terms (Thompson and Newmaster 2014) and they are becoming obtainable more easily in GenBank (Alström *et al.* 2013). Since most of the available sequences in GenBank are already published or analysed, they can be used in other context or to reproduce the phylogenies produced by other researchers. The data acquired from molecular examinations is (generally) objective. In looking at homologous characters from two taxa, there is typically no vagueness as to their differences and similarities. One essentially records the contrasts between two linear organised arrangements. One does not have to stress over choosing characters or making emotional decisions about when a character is in an alternate state. One may pick which molecule(s) to contemplate contingent on the specific issue. If one wishes to break down huge phylogenetic separations, a generally gradually evolving character is picked, for example, cytochrome c or rRNA. Then again, if closely related taxa are under investigation, a quick evolving character, for example, a pseudogene might be the character of decision. Certain drawbacks such as introgression and incomplete lineage sorting may arise in taxonomic inference between mitochondrial and nuclear genes

(Harrington *et al.* 2012). Therefore, one or more unlinked characters should be sampled, and morphological characters and/or geographic distributions should be used to complement the phylogeny (Puillandre *et al.* 2012).

1.4.1.3 Vocalisations

Another form of behavioural data is vocalisation, which can be used to serve many purposes, including mating rituals, alarm and contact calls, navigation to areas of nourishment sources, and social learning. In various species, male individuals perform songs amid mating ceremonies as a type of rivalry against different males and to attract females. Examples of other research on vocalisation incorporate insects (Robillard and Desutter-Grandcolas 2004), mammals (Cap *et al.* 2008) and birds (Gill 2007; Mennill *et al.* 2018; Mortimer *et al.* 2018). Vocalisation has also been used in systematics as a tool to: discover new species (Zimmer *et al.* 2001); assessment of taxonomic ranks (Whitney *et al.* 2000; Tobias *et al.* 2010; Seneviratne *et al.* 2012) and inferring relationships (Voelker 1999; Zimmer 2008; Robin *et al.* 2010; Mandiwana-Neudani *et al.* 2014).

The use of vocal characters from a systematics point of view has been unpopular (McCracken and Sheldon 1997; Catchpole and Slater 2008) compared to the rate at which nucleic acid characters continue to be used in avian systematics (Felsenstein 1984; Sayers *et al.* 2009). The challenge is that different types of data present their own inherent problems in phylogenetics and vocal characters are no exception. One of the reasons for the disinclination by systematists to vocalisation is that vocal characters may be susceptible to convergent evolution, making it difficult to differentiate between genetic and ecological components (McCracken and Sheldon 1997). In terms of the significance of using vocalisation to infer phylogenetic information, several studies have concurred to the worth of vocal characters as phylogenetic informative (Alström 2001; Navarro-sigüenza and Peterson 2004; Lei *et al.* 2005; Farnsworth and Lovette 2008; Cap *et al.* 2008; Mandiwana-Neudani *et al.* 2014). However, some authors have warned that vocalisations should be treated with greater attention and the methodology for using these in systematics needs to be refined and standardised (Alström and Ranft 2003). Alström and Ranft (2003) further stated that "sounds alone should not be used in making taxonomic decisions" but these can be a first pointer to the field of ornithology to gather

additional evidence such as further morphological, DNA or behavioural data, that can then be used in conjunction in taxonomic revisions (Krabbe and Cadena 2010; Isler *et al.* 2013).

1.4.2 Tracing the evolution of characters

In another avenue, researchers can map geographic histories of taxa and trace the history of characters and map them on phylogenies. Variations that may arise in characters can be inferred between organisms and their ancestors even if direct observations of those ancestors were not conducted (Maddison and Maddison 2000). There are various methods employed to trace character evolution and to map them on phylogenies, each composed of assumptions, advantages and shortcomings (Ho and Jermiin 2004). Ancestral reconstruction may help systematists to denote the biogeographic dispersal of species, test why and how characters evolved (Schaefer *et al.* 2012).

1.5 Sparse versus dense sampling in the systematic study

In systematics, conclusions may be drawn irrespective of the level of sampling approach and the number of characters used. Ames (1971) examined only nine species of larks in his study and concluded that all larks lack a pessulus, citing the work in MacGillivray's (1839) book, even though the latter study did not focus entirely on the structure of the lark syrinx. The issue can be centralised on the "phylogenetic representativeness" of a given taxonomic group. Sampling is an important step in scientific inquiry in order to reach well-informed conclusions. Therefore, browsing through studies that dealt with how often incorrect or biased taxon sampling is hypothesized is very important (Ilves 2008; Jenner *et al.* 2009; Palero *et al.* 2008; Ruiz *et al.* 2009; Tsui *et al.* 2009; Whitehead 2009). Plazzi *et al.* (2010) stated that "phylogenetic representativeness is a guarantee of a good and wise taxonomic coverage of the ingroup, but evidently it is not guaranteed of a good and robust phylogeny *per se*", which implies that the number of taxa included in a study may not necessarily influence the outcome of the results. Whitney *et al.* (1995), disregarded the use of morphological features for *Hylopezus nattereri* and focussed only on vocalisation of the species without presenting the morphological characters of the bird singing. Their validation and elevation of an individual to species rank using vocalisation

without morphological features resulted in treating *H. ochroleucus* as a conspecific with *H. nettereri* which was later discovered to be incorrect when including molecular markers (Carneiro and Aleixo 2014).

Another example is in Raposo and Höfling (2003) where they show the danger of generalising findings of the notion that Suboscine birds do not learn their vocalisation (e.g. Kroodsma 1982, 1984), while Snow (1970) indicated that young Suboscines males of *Procnias averano* learn their songs from males. Therefore, Raposo and Höfling (2003) concluded that Suboscines do not learn their song because their songs are inherited, based on the reference from the study of only three species of tyrannids that do not learn their songs: *Empidonax alnorum; E. traillii* (both in Kroodsma 1984) and *Sayornis phoebe* (Kroodsma and Konishi 1991). It was frequently inferred based on these examinations that Suboscines were unfit to learn their songs. In a recent study of Spotted antbirds (*Hylophylax naevioides),* a Suboscine group of birds that was subjected to isolation during its growth, showed that its vocalisation did not differ significantly from the adult in the wild which implied that these birds did not need to learn their species-typical song (Touchton *et al.* 2014).

1.6 Purpose of the study

1.6.1 Study Aim

The aim of this study was to investigate the utility of the syringeal and vocal characters in studying the evolutionary history of species of larks based on Alström *et al.*'s (2013) phylogenetic hypothesis.

1.6.2 Study objectives

The objectives of this study were outlined as follows:

i) to assess the distinctiveness of the three major clades (A - Alaudid, B – Mirafrid, C - Ammomanid) circumscribed in Alström *et al.* (2013).
ii) to produce comprehensive descriptions of the syringeal structures of selected lark species.

iii) to compare the syrinx of the selected lark species classified in clade A (Alaudid), B (Mirafrid) and C (Ammomanid).
iv) to produce comprehensive descriptions of the songs and characterise the study species vocally.
v) to reconstruct the vocal phylogeny.
vi) to trace the evolution of vocal and syringeal characters on the molecular phylogeny.

1.6.3 Study research questions

The following questions were set out in this study:

i) can the structure of syringes and songs of larks be used to assess the distinctiveness of the three circumscribed major clades (A – Alaudid, B – Mirafrid, C – Ammomanid) in Alström *et al.* (2013)?

ii) how does the syringeal structure of the selected lark species compare in clade A (Alaudid), B (Mirafrid) and C (Ammomanid)?

iii) can songs be used to characterise the species of larks?

iv) how does the vocal phylogeny of larks compare to the molecular phylogeny?

v) how did the syringeal and song characters of larks evolve?

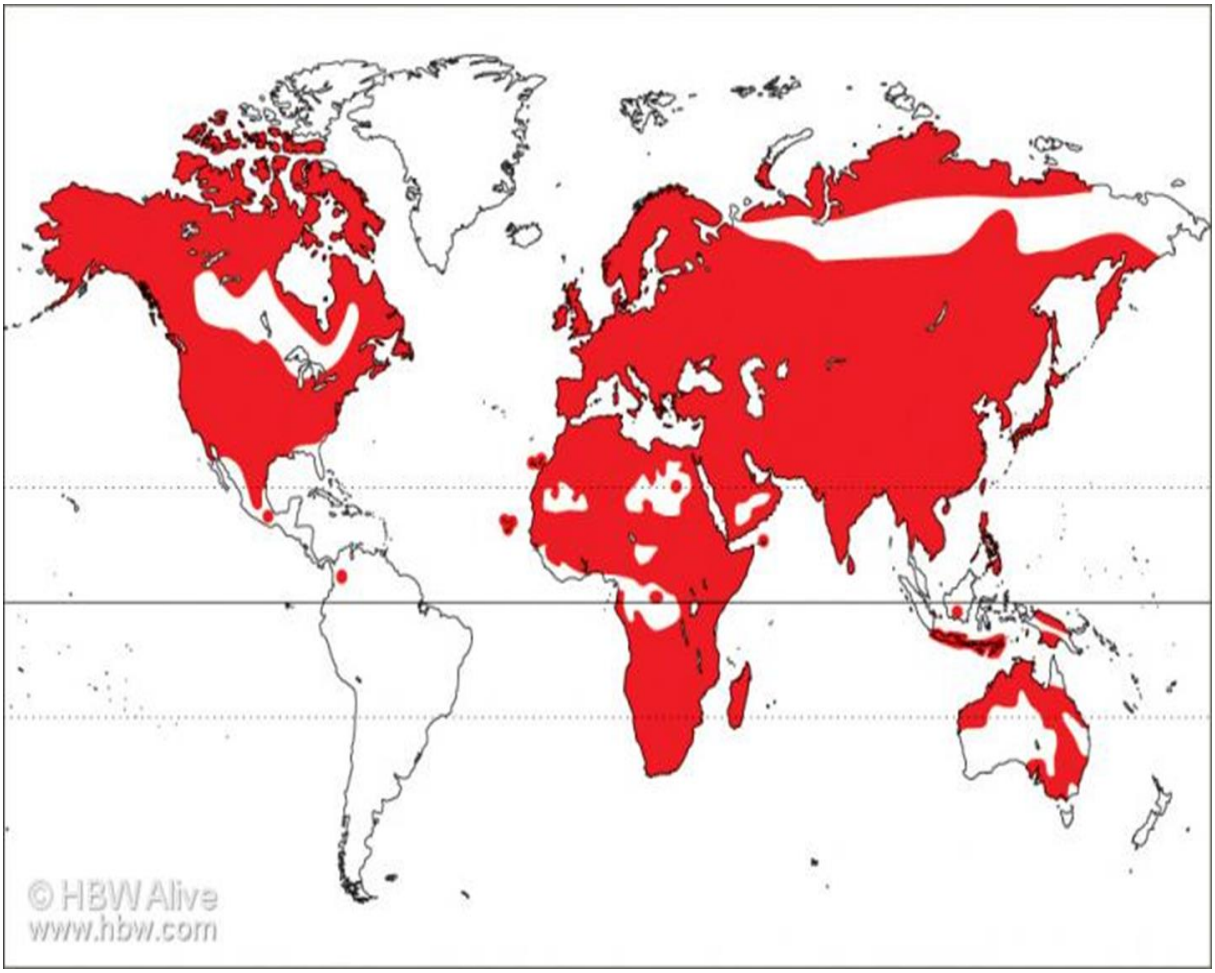


FIGURE 1.1. MAP SHOWING THE GLOBAL DISTRIBUTION OF LARKS (ALAUDIDAE). SOURCED FROM DE JUANA *ET AL*. (2020).

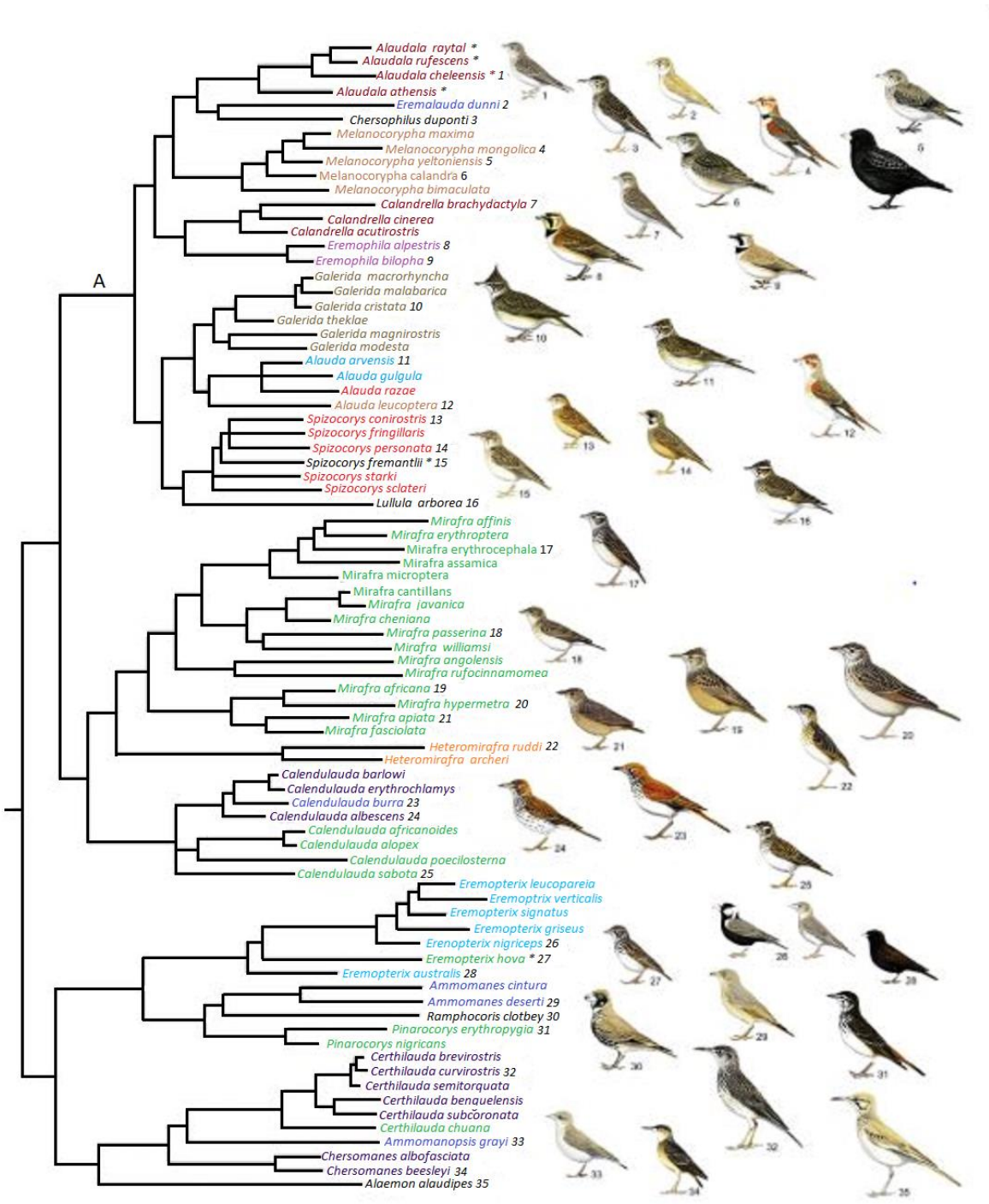


FIGURE 1.2. PHYLOGENETIC TREE OF THE LARK FAMILY, ALAUDIDAE AS SOURCED FROM ALSTRÖM *ET AL.* (2013). DIFFERENT COLOURS OF NAMES INDICATE GENERA AS DEFINED BY PETERS (1960) BASED ON MORPHOLOGY; MONOTYPIC GENERA ARE SHOWN IN BLACK. REVISED NAMES COMPARED TO GILL AND DONSKER (2012) ARE INDICATED BY *.

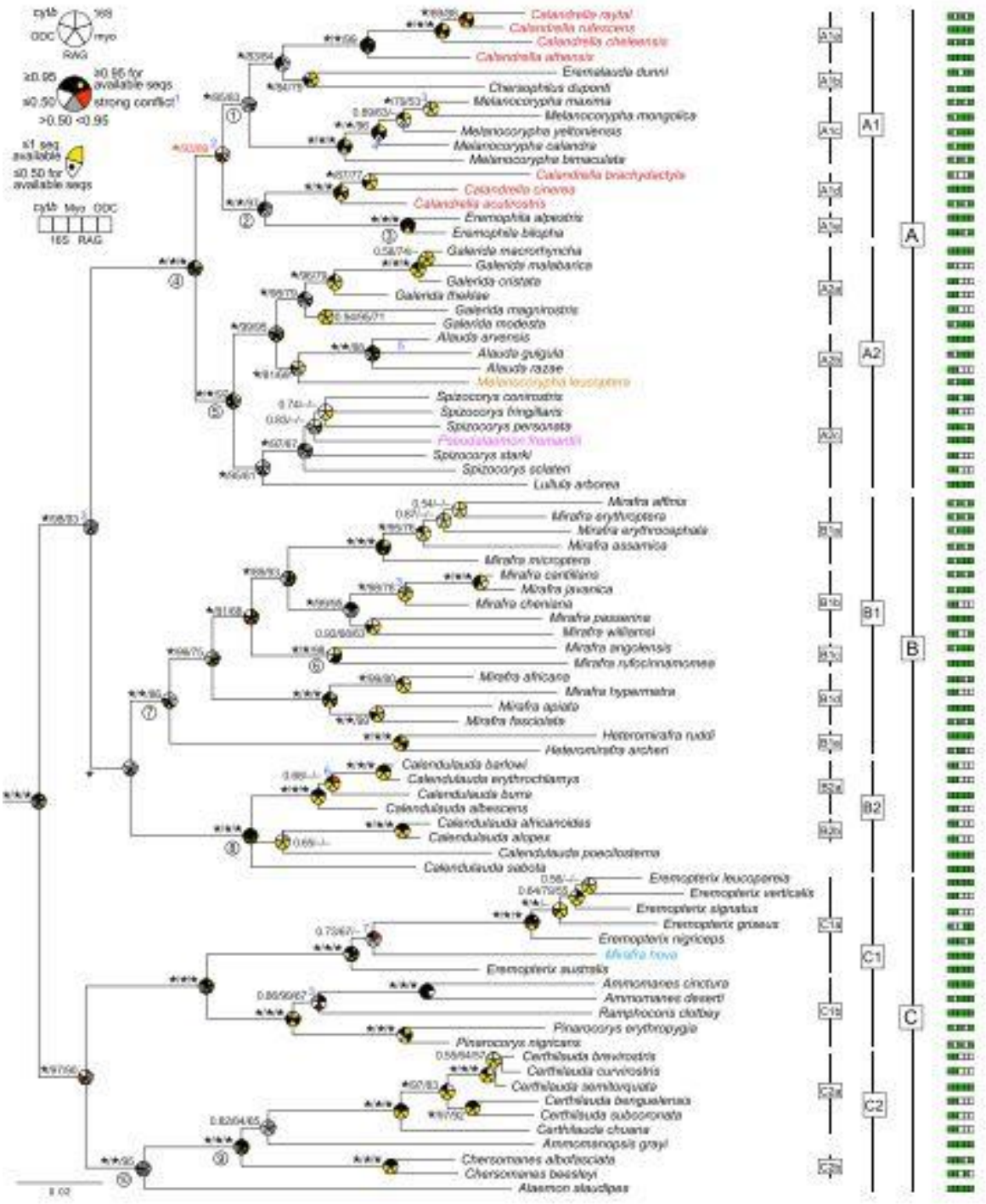


FIGURE 1.3. PHYLOGENETIC TREE OF THE LARK FAMILY, ALAUDIDAE AS SOURCED FROM ALSTRÖM *ET AL.* (2013). MAJORITY RULE (50%) CONSENSUS TREE OF ALAUDIDAE BASED ON CONCATENATED NUCLEAR ODC, MYOGLOBIN AND RAG1 + 2 AND MITOCHONDRIAL CYTOCHROME *B* (*CYTB*) AND 16S SEQUENCES, INFERRED BY BAYESIAN INFERENCE, ANALYSED IN FIVE PARTITIONS.

APPENDIX

APPENDIX 1.1. LIST OF SPECIES OF LARKS OF THE WORLD. ENGLISH AND SPECIFIC NAMES ARE AS PROPOSED IN THE IOC WORLD BIRD LIST (GILL AND DONSKER 2020). THE CLADES FOLLOW THE CIRCUMSCRIPTION IN ALSTRÖM *ET AL.* (2013). '*' DENOTES THE SPECIES THAT WERE NOT INCLUDED IN ALSTRÖM *ET AL.* (2013).

Clade A	English name	Scientific name
*	Obbia Lark	*Spizocorys obbiensis* Witherby, 1905
	Sclater's Lark	*S. sclateri* (Shelley, 1902)
	Stark's Lark	*S. starki* (Shelley, 1902)
	Short-tailed Lark	*S. fremantlii* (Lort Phillips, 1897)
	Masked Lark	*S. personata* Sharpe, 1895
	Botha's Lark	*S. fringillaris* (Sundevall, 1850)
	Pink-billed Lark	*S. conirostris* (Sundevall, 1850)
	White-winged Lark	*Alauda leucoptera* Pallas, 1811
	Raso Lark	*A. razae* (Alexander, 1898)
	Oriental Skylark	*A. gulgula* Franklin, 1831
	Eurasian Skylark	*A. arvensis* Linnaeus, 1758
	Sykes's Lark	*Galerida deva* (Sykes, 1832)
	Sun Lark	*G. modesta* Heuglin, 1864
	Large-billed Lark	*G. magnirostris* (Stephens, 1826)
	Thekla's Lark	*G. theklae* Brehm, AE, 1857
	Crested Lark	*G. cristata* (Linnaeus, 1758)
	Malabar Lark	*G. malabarica* (Scopoli, 1786)
	Maghreb Lark	*G. macrorhyncha* Tristram, 1859
	Horned Lark	*Eremophila alpestris* (Linnaeus, 1758)
	Temminck's Lark	*E. bilopha* (Temminck, 1823)
	Hume's Short-toed Lark	*Calandrella acutirostris* Hume, 1873
	Mongolian Short-toed Lark	*C. dukhunensis* (Sykes, 1832)
	Blanford's Lark	*C. blanfordi* (Shelley, 1902)
*	Rufous-capped Lark	*C. eremica* (Reichenow and Peters, JL, 1932)
	Red-capped Lark	*C. cinerea* (Gmelin, JF, 1789)
	Greater Short-toed Lark	*C. brachydactyla* (Leisler, 1814)
	Bimaculated Lark	*Melanocorypha bimaculata* (Ménétries, 1832)
	Calandra Lark	*M. calandra* (Linnaeus, 1766)
	Black Lark	*M. yeltoniensis* (Forster, JR, 1768)

	Mongolian Lark	*M. mongolica* (Pallas, 1776)
	Tibetan Lark	*M. maxima* Blyth, 1867
	Dupont's Lark	*Chersophilus duponti* (Vieillot, 1824)
	Dunn's Lark	*Eremalauda dunni* (Shelley, 1904)
	Athi Short-toed Lark	*Alaudala athensis* (Sharpe, 1900)
	Asian Short-toed Lark	*A. cheleensis* Swinhoe, 1871
*	Somali Short-toed Lark	*A. somalica* Sharpe, 1895
	Lesser Short-toed Lark	*A. rufescens* (Vieillot, 1819)
	Sand Lark	*A. raytal* (Blyth, 1845)
	Woodlark	*Lullula arborea* (Linnaeus, 1758)
Clade B	**English name**	**Scientific name**
	Sabota Lark	*Calendulauda sabota* (Smith, A, 1836)
	Pink-breasted Lark	*C. poecilosterna* (Reichenow, 1879)
	Foxy Lark	*C. alopex* (Sharpe, 1890)
	Fawn-colored Lark	*C. africanoides* (Smith, A, 1836)
	Karoo Lark	*C. albescens* (Lafresnaye, 1839)
	Red Lark	*C. burra* (Bangs, 1930)
	Dune Lark	*C. erythrochlamys* (Strickland, 1853)
	Barlow's Lark	*C. barlowi* (Roberts, 1937)
	Rudd's Lark	*Heteromirafra ruddi* (Grant, CHB, 1908)
	Archer's Lark	*H. archeri* Clarke, S, 1920
	Eastern Clapper Lark	*Mirafra fasciolata* (Sundevall, 1850)
	Cape Clapper Lark	*M. apiata* (Vieillot, 1816)
	Red-winged Lark	*M. hypermetra* (Reichenow, 1879)
	Rufous-naped Lark	*M. africana* Smith, A, 1836
	Flappet Lark	*M. rufocinnamomea* (Salvadori, 1865)
	Angolan Lark	*M. angolensis* Barboza du Bocage, 1880
	Williams's Lark	*M. williamsi* Macdonald, 1956
	Monotonous Lark	*M. passerina* Gyldenstolpe, 1926
	Melodious Lark	*M. cheniana* Smith, A, 1843
	Horsfield's Bush Lark	*M. javanica* Horsfield, 1821
	Singing Bush Lark	*M. cantillans* Blyth, 1845
	Burmese Bush Lark	*M. microptera* Hume, 1873
	Bengal Bush Lark	*M. assamica* Horsfield, 1840
	Indochinese Bush Lark	*M. erythrocephala* Salvadori and Giglioli, 1885
	Indian Bush Lark	*M. erythroptera* Blyth, 1845
	Jerdon's Bush Lark	*M. affinis* Blyth, 1845
	Gillett's Lark	*M. gilletti* Sharpe, 1895
	Rusty Bush Lark	*M. rufa* Lynes, 1920
	Collared Lark	*M. collaris* Sharpe, 1896
	Ash's Lark	*M. ashi* Colston, 1982
	Somali Lark	*M. somalica* (Witherby, 1903)

	Friedmann's Lark	*M. pulpa* Friedmann, 1930
	Kordofan Lark	*M. cordofanica* Strickland, 1852
	White-tailed Lark	*M. albicauda* Reichenow, 1891

Clade C	English name	Scientific name
	Greater Hoopoe-Lark	*Alaemon alaudipes* (Desfontaines, 1789)
	Lesser Hoopoe-Lark	*A. hamertoni* Witherby, 1905
	Beesley's Lark	*Chersomanes beesleyi* Benson, 1966
	Spike-heeled Lark	*C. albofasciata* (Lafresnaye, 1836)
	Gray's Lark	*Ammomanopsis grayi* (Wahlberg, 1855)
	Short-clawed Lark	*Certhilauda chuana* (Smith, A, 1836)
	Karoo Long-billed Lark	*C. subcoronata* Smith, A, 1843
	Benguela Long-billed Lark	*C. benguelensis* (Sharpe, 1904)
	Eastern Long-billed Lark	*C. semitorquata* Smith, A, 1836
	Cape Long-billed Lark	*C. curvirostris* (Hermann, 1783)
	Agulhas Long-billed Lark	*C. brevirostris* Roberts, 1941
	Dusky Lark	*Pinarocorys nigricans* (Sundevall, 1850)
	Rufous-rumped Lark	*P. erythropygia* (Strickland, 1852)
	Thick-billed Lark	*Ramphocoris clotbey* (Bonaparte, 1850)
	Desert Lark	*Ammomanes deserti* (Lichtenstein, MHK, 1823)
	Bar-tailed Lark	*A. cinctura* (Gould, 1839)
	Rufous-tailed Lark	*A. phoenicura* (Franklin, 1831)
	Black-eared Sparrow-Lark	*Eremopterix australis* (Smith, A, 1836)
	Madagascan Lark	*E. hova* (Hartlaub, 1860)
	Black-crowned Sparrow-Lark	*E. nigriceps* (Gould, 1839)
*	Chestnut-backed Sparrow-Lark	*E. leucotis* (Stanley, 1814)
	Ashy-crowned Sparrow-Lark	*E. griseus* (Scopoli, 1786)
	Chestnut-headed Sparrow-Lark	*E. signatus* (Oustalet, 1886)
	Grey-backed Sparrow-Lark	*E. verticalis* (Smith, A, 1836)
	Fischer's Sparrow-Lark	*E. leucopareia* (Fischer, GA and Reichenow, 1884)

CHAPTER 2

Gross morphological and histological description of the syrinx of larks (Passeriformes, Alaudidae)

2.1 Introduction

2.1.1 What is a syrinx?

The vocalisations in mammals and birds, in particular, are controlled by different vocal organs, namely, the larynx and the syrinx, respectively (Tsukahara *et al.* 2008). The process of sound production in birds can be described as interactions between muscles which influences airflow that initiates tissue vibrations in the syrinx (King and McLelland 1984). The syrinx is not only responsible for production of vocalisations but can be used for sex determination, classification of birds, and for determining phylogenetic positions (Gaban-Lima and Höfling 2006; Mandiwana-Neudani *et al.* 2011). The avian trachea consists of complete rings (King and McLelland 1984) which in various species become ossified to various extent (Piperno and Peirone 1975). At the level where the syrinx is located, the trachea branches into two primary bronchi (Dyce *et al.* 1996), and thin membranes in the syrinx named *membrana tympaniformis lateralis* and *medialis* responsible for sound production (Baumel *et al.* 1993). There are three known types of the syrinx that have been described based on the location relative to the trachea and the bronchi. In the case where the syrinx is located at the end of the trachea, it is termed '*syrinx trachealis*', where it is below the bifurcation point it is termed '*syrinx bronchialis*' and the one located between the trachea and bronchi is termed '*syrinx tracheo-bronchialis*' (Ames 1971; King 1989; Baumel *et al.* 1993).

The majority of passerine birds have the '*syrinx tracheo-bronchialis'* type and in some non-passerines such as the owls, cuckoos and nightjars (Ames 1971), the syrinx may be of the '*syrinx bronchialis*' type. Among the passerines, there are species such as Furnariidae (ovenbirds), Dendrocolaptidae (woodcreepers), Formicariidae (ground antbirds), Thamnophilidae (typical antbirds), Rhinocryptidae (tapaculos), and

Conopophagidae (gnateaters) which fall under Suboscine Passeriformes that possess a '*syrinx trachealis*' type while the Oscines passerines have the '*syrinx tracheo-bronchialis'* type (Irestedt *et al.* 2002).

The general structure of the Oscine passerine syrinx comprises the *tympanum*, an unpaired ossified cylinder located at the caudal end of the trachea, formed by the close apposition or fusion of four to six tracheal rings and one paired half-ring, and the *pessulus*, unpaired ossified cartilage, located at the caudal end of the *tympanum* derived from the fusion of two bronchial half-rings. Lateral and medial tympaniform membranes serve as sound generators through vibration and contribute to the anatomy of the syrinx by connecting its muscles (see Fig. 2.1; Suthers 2004).

Apart from the various types of syrinx and muscle attachments, vocalisations produced by passerine birds divide them into Oscine birds (those that produce complex songs and are in the suborder Passeri) and Suboscine birds (they produce simple vocalisations and are in the suborder Tyranni) (Frank *et al.* 2006). The reason towards this phenomenon is hypothesised to be the number of syringeal muscles involved in the formation and structure of the syrinx. For example, Zebra Finch *Taeniopygia* guttata, an Oscine passerine bird, has complex vocalisations while the African Broadbill *Smithornis capensis,* a Suboscine passerine bird, produces simple vocalisations.

In contrast to the Passeriformes that produce songs, non-passerines typically produce vocalisations referred to as calls. The Red Junglefowl *Gallus gallus* (non-passerine), for example, has three pairs of tracheal muscle and produces simple calls (Gaunt and Gaunt 1977). The majority of Oscine species have five pairs of syringeal muscles, and almost all of them have complex songs (Ames 1971) e.g. Cardinalidae (Suthers *et al.* 1999), but there is exception e.g. in corvids (Corvidae). These birds have seven pairs of syringeal muscles and produce poor songs but may render various calls (Kuroda 1990). On the other hand, larks, however, have fewer number of muscles but are able to produce complex songs. Therefore, suggesting that the number of syringeal muscles is related to the type of vocalisations produced should be dealt with scrutiny.

There is a need for the further investigation on the detailed functioning of each syringeal component as to whether the production of complex vocalisation is dependent

on the number of muscles or syringeal complexity. The syrinx of Cockatiels *Nymphicus hollandicus,* non-passerines in the order Psittaciformes have three pairs of syringeal muscles and two pairs of tracheal muscles (Larsen and Goller 2002), and their vocalisation includes mimicry (Cruickshank *et al.* 1993). Hence, some species from this order can even talk and produce complex vocalisations despite not being Oscine birds, e.g. Grey Parrot *Psittacus erithacus*. Nevertheless, the musculature of the syringes contributes largely to voice production (Gaunt and Gaunt 1985; King 1989) and has been found to play a significant role in the classification of birds (Ames 1971; Beddard and Parson 1893; Prum 1992). This view is supported by studies of the syringes of the Red Junglefowl *Gallus gallus* (Myers 1917), the male Mallard Duck *Anas platyrhynchos* (Frank *et al.* 2006) and the Sage Grouse *Centrocercus urophasianus* (Krakauer *et al.* 2009).

Some syringeal components responsible for sound production are said to be driven by sexual selection and change between related species. Therefore, the syrinx of males should differ from that of females in some bird species. The size of the male syrinx in European Starling *Sturnus vulgaris* is known to be larger than that of females, since males have complex songs compared to the slow and short repertoires rendered by females (Prince *et al.* 2011). A few studies found the male syrinx to be larger than females in Zebra Finch *Taeniopygia guttata* (Luine *et al.* 1980; Wade and Buhlmann 2000; Wade *et al.* 2002; Veney and Wade 2004; Veney and Wade 2005), and Riede *et al.* (2010) showed that differences in the vibrating tissues and cartilaginous framework are consistent with the production of a greater range of sound frequencies in males than females. In contrast to this, Appel (1929) found no sexual dimorphism in the structure of syrinx of the Red Junglefowl *Gallus gallus*. In most Oscines birds, the fiber type composition of syringeal muscles showed no sexual dimorphism (Uchida *et al.* 2009; Christensen *et al.* 2014; Christensen *et al.* 2017).

The syrinx is an anatomically complex organ and it is interspecifically diverse even in species that lack special structures. Thus, syringeal morphology has proven to be informative in some systematic studies of birds (Ames 1971, Gaban-Lima and Höfling 2006; Zimmer *et al.* 2008; Mandiwana-Neudani *et al.* 2011). The utility of syringeal characters in Passeriformes for phylogenetic analysis has been scarce, but Prum (1992)

studied the syringeal characters of Manakins (Pipridae) and found them to be autapomorphic in several clades.

2.1.2 The history of syrinx morphology

Herissant (1753) was the first to describe the syrinx as the source of the voice in a non-passerine, the domestic duck (Anseriformes). The passerine syrinx was first described by Vicq D'Azyr (1779), who noted that it was represented by the simplest form, but later was found to be more complex when Cuvier (1802) studied the syrinx of the European Starling. The muscles of the syrinx of songbirds were described in more detail by Savart (1826) while the application of syringeal morphology to the classification of birds was first attempted by Nitzsch (1829). Although not very successful at using it as a tool to classify birds, Nitzsch noted singing birds had a strongly muscled syrinx. Blyth (1838) examined the vocal organ in cotingas (Cotingidae), manakins, and tyrant flycatchers (Tyrranidae) of the neotropics and concluded that it was as complex as in European passerines.

MacGillivray (1838) described the syringeal structures in thirty-nine Oscine genera, observed the syrinx of *Tyrannus*, *Myiarchus*, *Contopus* and *Empidonax* and concluded that the genus *Tyrannus* lacks a pessulus. Eyton (1841-1844) was the first to describe the "trachealis" syrinx. He was primarily interested in the *musculus sternotracheales*, a muscle that extends from the sternum to the trachea, and in many cases, he described the syrinx based on these muscles.

Müller (1847, 1878) was the first to examine the syringes of more genera of birds than anyone before him. This work provided a framework based on syringeal morphology for the systematic arrangement of the Passeriformes and associate the form of the syrinx to other anatomical characters, especially a scutellate tarsus. His work was considered the foundation for all classifications in which the syrinx has been utilised as a taxonomic character.

Herre (1859) and Owen (1866) contributed additional information on the syringeal structure of many European passerines. Huxley (1877) introduced the term "syrinx" to replace "upper" and "lower" larynx as it was named until then. Garrod (1876,1877) worked on the syrinx of many non-Oscine genera such as *Menura, Atrichornis, Pitta* and several

Tyrant flycatchers. He referred to the insertion of the intrinsic muscles as either “mesomyodian” which means having the middle of the half/semi-rings (incomplete rings on bronchial tubes of the syrinx) attached to the intrinsic muscle, or “acromyodian” meaning such birds have the intrinsic muscle attached to the ends of the bronchial semi-rings.

Furthermore, Wunderlich (1886) focused on describing the syrinx of European birds, and he contributed greatly towards the embryology of the syrinx, which he depicted in the domestic duck and the House Sparrow. Furbringer (1888) summed up the work of previous authors and for the condition in which the muscles insert in both ends of a single element, he coined the word “diacromyodian”.

Through the collection of the findings of the authors mentioned, Gadow and Selenka (1893) described the syrinx of the Carrion Crow (*Corvus corone*). Haecker (1900) demonstrated differences in muscles and cartilages between sexes and age groups in many European passerine species. The syringeal structure of Palearctic songbirds in which the focus was on the individual variation of the syrinx within species was examined by Setterwall (1901). The author was interested in the interior of the syrinx, especially small cartilaginous elements. His theories on the function of the syrinx later influenced the studies by Rüppell (1933) and Greenewalt (1968).

In just over a century since the discovery of the syrinx, including its description in the systematic morphology of birds was a common practice. Pycraft (1905) remarks that the syrinx of the Wrenthrush *Zeledonia coronata* is “typically Oscine”. During this era, many passerines were classified mainly based on the syringeal structure. Bates (1914) removed *Smithornis* from the Oscine family Muscicapidae utilising the morphology of the syrinx. In the earlier works, the broadbills were placed near Caprimulgidae, in or near Coraciidae, in the Todidae, Muscicapidae, Pipridae or Cotingidae (Sclater 1872), followed by Lowe (1924) ten years later who compared the syrinx of *Smithornis rufolateralis* with *Eurylaimus,* resulting in *Smithornis* being placed in the Eurylaimidae. *Smithornis* was recently moved to Calyptomenidae (Selvatti *et al.* 2017). In Lowe (1931), *Pseudocalyptomena* was placed in the Eurylaimidae based on the syrinx and other characters.

The utilisation of syringeal morphology has seen several passerine genera moved from one family to another in the twentieth century. The genera *Melampitta* (Mayr 1931), *Lawrencia* (Wetmore and Swales 1931) and *Ramphocaenus* (Wetmore 1943) were shown to be Oscine. Genus *Psilorhamphus*, considered to be a close relative of *Ramphocaenus*, was proved to belong to the Rhinocryptidae, which is a family of furnarioids (Plotnick 1958). The relationship between the Madagascar genus *Neodrepanis* and the peculiar Asities (Philepittidae) was established through syringeal morphology by Amadon (1951). The close affinity of the Cracticidae to the Corvidae rather than to the Laniidae was established after Mayr (1931) compared the syrinx of *Gymnorhina* with that of *Corvus.* The syrinx of the African River Martin *Pseudochelidon eurystomina* was studied by Mayr and Amadon (1951) resulting in it being placed in its own subfamily within the Hirundinidae.

The intrinsic syringeal muscles were described for the first time in Rüppell (1933) when studying the syrinx of the *Lepidocolaptes* sp*.* The Sharpbill *Oxyruncus cristatus* was considered a modified tyrannid following Clark (1913) who compared its syrinx with that of the tyrannid Black Phoebe *Sayornis nigricans.* By utilising only, the morphology of the syrinx of plantcutters *Phytotoma* spp*.,* Kuchler (1936) used that genus to relate it to Cotingidae. Lanyon and Lanyon (1989) later utilised electrophoretic, syringeal, and osteological characters to investigate phylogenetic in genus *Phytotoma*. In the latter study, the syringeal as well as the biochemical characters support that plantcutters should be within the cotingid genus *Ampelion*. Earlier, in some Pipridae, the evolutionary relationships were established from the syringeal morphology (Lowe 1942; Ericson *et al.* 2006).

Miskimen's (1951) seminal work on the passerine syrinx investigated the vocal organs of twenty-nine Oscine species and two tyrannids. The results of the study differed from Wunderlich (1886), Haecker (1900), Setterwall (1901) and Köditz (1925), all of whom studied Old World species, as the findings indicated that the number of syringeal muscles in North American songbirds ranged from four to seven pairs. Miskimen was able to confirm that sound is produced during the expiration cycle of respiration using the replication experiment by Rüppell (1933), also indicating that the me*mbrana semilunaris* does not play a major role in sound production. Miskimen (1951) compared the structure

of syrinx across many passerine species, including Horned Lark *Eremophila alpestris,* wherein six pairs of muscles were observed and concluded that “in general the birds possessing more muscles can produce a wider variety of notes”. Miskimen (1963) described the syrinx in six genera of the Tyrannidae providing a detailed report on the tyrannid syrinx including the oblique character of the ventral intrinsic muscles. The oblique muscles attached to the ventral or lateral side of the syrinx, distinguishable by the fibre-direction’ that were observed in various taxa were used in classification. The ventral muscles (*musculus syringeo-ventralis*) were analysed based on its origin and insertion on the bronchial ring and the presence of lateral muscles (*musculus syringeo-lateral*).

Regarding the function of syringeal structures in sound or song production, both Greenewalt (1968) and Stein (1968) analysed several vocalisations of a wide range of birds, including both non-passerines and passerines, and proposed various hypotheses for the mechanism of sound production and modulation. Both authors independently developed models of avian sound development combining the ability of two separate acoustic sources inside the syrinx to make sounds. Chamberlain *et al.* (1968) studied the syrinx of American Crow *Corvus brachyrhynchos*, with special attention on the action of muscles and their effect on the syringeal membranes. He found the American Crow morphologically able to produce a wide number of notes. Later, the investigation by Elemans *et al.* (2009) found that sound creation in birds was driven by the thin medial tympaniform membrane which was also studied by King (1989). Until the rise of molecular biology, syringeal diversity was a significant instrument to help systematists and taxonomists in avian classification (Ames 1971). Syringeal morphology has been studied intensively (King 1989; Düring *et al.* 2013).

2.1.3 Errors noted in previous studies in the anatomy of the syrinx

According to Bock (1972), a central problem with important theoretical and practical overtones is homologising the tracheal and bronchial rings. Earlier researchers did not delve into this matter and numbered the rings from the tracheal-bronchial junction. Ames (1971) avoided this question, apart from stating that he found the theoretical tracheal-bronchial numbering unreliable and preferred to number the rings from a junction in the bronchus between an anterior "type A" ring and a posterior "type B" ring. This terminology

problem was deemed serious because Ames (1971) described the attachment of syringeal muscles in terms of "A" and "B" rings. He did not attempt to ascertain whether the A-B junction is homologous in passerine birds and whether individual rings are homologous in these birds. Nor did he attempt to ascertain whether correlations exist between these rings and other syringeal features, e.g. the tympaniform membranes or the labium. This problem has led several researchers to number the tracheal and bronchial rings differently, hence, resulting in distinct descriptions of the syrinx. This seemed to make a case to revise and standardise the nomenclature of anatomical structures of the syrinx. However, King (1989) and Düring *et al.* (2013) presented similar nomenclature of anatomical structures of the syrinx. The bronchial rings were labelled as Type B rings and the tracheal rings as Type T rings. This makes it easier to differentiate between the different rings of the syringes by labelling bronchial rings as “B” and tracheal rings as “T”.

2.1.4 What do we know about the syrinx of larks?

2.1.4.1 Background information about the focal species

The larks (Passeriformes) belong to an ancient and highly distinct family of Oscine passerines, the Alaudidae with no near relatives (Alström *et al.* 2013) and they are deemed to have a sister relationship with all Sylvioidea (Alström *et al.* 2006). They are distributed on all continents except Antarctica and Oceania (New Zealand, specifically) with all the genera being present in Africa, and thirteen in Eurasia. The New World holds one species, the Horned Lark *Eremophila alpestris,* Madagascar holds only Madagascan Lark *Eremopterix hova* and Australia hosts a single species Horsfield's Bush Lark *Mirafra javanica* (de Juana *et al.* 2020). Larks are found in a wide range of environments including open arid, semi-arid, and open mesic grasslands and woodland in a variety of biomes. According to Donald (2004), their choice of habitat is strongly correlated with plumage pattern and the plumage colouration follows Gloger´s rule (Delhey 2019), i.e. birds in moist areas tend to be darker than those found in arid areas. Therefore, larks are generally cryptic in appearance and have an interesting range of sexual size dimorphism

with males, averaging 20-25% larger than females (de Juana *et al.* 2020) and pronounced sexual dichromatism in most members of the genus *Eremopterix*.

2.1.4.2 The syrinx and the classification of larks

Larks are separated from other Oscine passerine birds by the structure of their syrinx which is deemed to lack a pessulus (Mayr and Amadon 1951; Ames 1971; King and McLelland 1984). In the study, "The morphology of the syrinx in passerine birds", Ames (1971) studied extensively the suborder Passeres citing MacGillivray (1839) as the earliest study to give evidence that larks lack a pessulus in their syrinx. However, MacGillivray (1839) did not mention "pessulus". The author only included images that show the digestive tract of the Horned Lark *Eremophila alpestris.* Therefore, the consolidation of literature on the syrinx of larks to our knowledge points to Mayr and Amadon (1951) as being the earliest study that mentioned the lack of a pessulus in larks, unless otherwise, Ames (1971) had other sources not mentioned in his study.

The family was found to be less variable than the Tyranni or Furnaii in the syringeal structure. Ames (1971) suggested the family was narrowly monophyletic, as uniformity of the syrinx structure could be observed throughout the suborder despite its complex nature. Furthermore, it was stated that the absence of a pessulus in larks should not be viewed as a primitive state, for its absence should be considered almost secondary. The pessulus is present in most Suboscine passerines and the non-passerine orders Piciformes, Coraciiformes and Galliformes and was likely present in the ancestor of the Passeres.

In Ames (1971), the syrinx of the genus *Corvus* was considered a "typical Oscine syrinx" and was employed as a reference syrinx representing the syringeal structure of a large majority of Oscines. Lark species that were analysed in Ames (1971) can be categorised based on clades designated in Alström *et al.* (2013) (Fig. 1.2) as follows: clade A – hereafter the Alaudid: (*Alauda arvensis, Alaudala rufescens* (formerly: *Calandrella rufescens*), *Calandrella cinerea, Melanocorypha yeltoniensis, Galerida modesta* and *Galerida cristata*) and clade B - Mirafrid: (*Mirafra angolensis* and *Calendulauda sabota* (formerly: *Mirafra sabota*). There were no representatives of clade C (the Ammomanid). The above-mentioned species are North African/European species

except for *Calendulauda sabota*, *Mirafra angolensis* and *Calandrella cinerea* which are found either in the central or southern African regions.

Taking cognisance of the fact that the classical work in Ames (1971) precedes the availability of a comprehensive phylogeny of the Alaudidae, it is also apparent that the sampling coverage by Ames was skewed in terms of family, genera, and species representation. Further to this, the study lacked detailed descriptions of these syringes and the species analysed were largely North African/European species except for *C. sabota*, *M. angolensis* and *C. cinerea* which are sub-Saharan species. The syringeal morphology and other molecular studies denote that larks are an ancient and highly distinct family of Oscine passerines with 'no close relative' (Dean *et al.* 1992, Barker *et al.* 2002; Barnes 2007). The chapter about larks by de Juana *et al.* (2004) brought forth the fact that the syrinx, which is the organ of voice production, is relatively simple in structure having just five pairs of muscles and with a rudimentary pessulus (bony structure located at the bronchial junction). The challenge with this is that there is no citation to this notion or the examination of the syrinx of larks that was mentioned in this publication. Furthermore, the evidence towards this notion made in de Juana *et al.* (2004) should be found in Verheyen (1958) (as per personal communication between Dr Cecilia Kopuchian and Prof Eduardo de Juana in 2020) and a later study by Suárez *et al.* (2009). The latter cited de Juana *et al.* (2004) while Verheyen (1958) did not present neither the source of this notion nor the diagrams of the syrinx showing the rudimentary pessulus. On the other hand, according to Dean and Williams (2004), larks differ from other Oscine passerines in lacking an ossified pessulus.

The foregoing outline demonstrates considerable uncertainty and confusion about the structure of the syrinx of larks, first and foremost whether the syrinx of larks possesses a pessulus or not and whether the pessulus if present is ossified or not. These also highlight the issue of sparse versus dense sampling when doing research and the importance of verifying and correctly citing the findings or work that was published.

The focus in this chapter was on providing the description of the syrinx of larks by including a fair sample size of species. The present study is to our knowledge the first to analyse the histology of the syrinx of larks.

2.1.5 Aim

The aim of this chapter was to describe the syrinx of selected southern African lark species (Alaudidae) by determining its gross morphological and histological structure.

2.1.6 Objectives

To:

i) describe the gross morphological and histological structure of the syrinx of the larks.

ii) compare the syrinx of the selected lark species classified in clade A (Alaudid), B (Mirafrid) and C (Ammomanid).

2.2 Materials and Methods

2.2.1 Sampling of syringes

For comparative purposes, sampling also included the sampling of outgroup species. Although there is no consensus as to the outgroups of larks. Guidance was sought from the literature that covers studies of larks (Barnes 2007; Alström *et al.* 2013). This included members of the genera *Anthus*, *Cisticola*, *Hirundo*, *Prinia, Sylvia* and *Sylvietta.* This study covered a wide range of species, so fieldwork was conducted mainly in spring/summer (October to December 2016), during late summer/autumn (February to April 2017) and spring/summer (October to December 2017) in Limpopo, Mpumalanga and Northern and Western Cape provinces.

Birds were shot using an air rifle or a special calibre shot gun used for collecting material for museum study skins. The sampling of larks proceeded upon approval of ethical clearance by the University of Limpopo's Animal Research Committee. Subsequently, the necessary collecting permits from various provinces where birds occur were acquired. The biometric data of the captured birds were recorded following the standard SAFRING bird ringing manual (de Beer *et al.* 2001). The specimens were frozen at - 20°C and stored until the syringes were excised to analyse the gross morphology of the syrinx. In the case of syringes that were analysed histologically, collected birds were dissected in the field. The excised syringes were pinned on a wax and transferred to

Karnovsky's (glutaraldehyde) fixative (Karnovsky 1965) to keep the shape of the syrinx intact. All the specimens from the field were transported to the departmental laboratory for further analyses.

2.2.2 Gross morphological and histological examination of syringes

2.2.2.1 Choice of taxa and preparation of syringes

The analysis of the gross morphology of syringes spanned nineteen (19) lark species representing eight genera and also five outgroup species (Table 2.1). The outgroup species were African Pipit *Anthus cinnamomeus,* Lesser-striped Swallow *Hirundo abyssinica,* Garden Warbler *Sylvia borin,* Long-billed Crombec *Sylvietta rufescens* and Rattling Cisticola *Cisticola chiniana* (Table 2.1)*.* After excising of the syringes, they were preserved in 10% buffered formalin for 5 days, then transferred to 70% ethanol for storage until they were subjected to further processing.

Regarding the histological component, four lark species and five outgroup species were examined (Table 2.2). The outgroup species were African Pipit *Anthus cinnamomeus,* Plain-backed Pipit *Anthus leucophrys,* Marsh Warbler *Acrocephalus palustris*, Rattling Cisticola *Cisticola chiniana* and Cape Long-claw *Macronyx capensis*.

All the syringes analysed in this study were obtained from adult birds to prevent any bias in the results which could arise as a result of life stage or any other developmental differences in the birds sampled. Where possible, several individual birds per species were analysed in order to study any intraspecific variation. Unfortunately, very few individuals were sexed, and this did not suffice for sexual variation to be studied.

2.2.2.2 Gross morphological analysis

Syringes were cleaned off of excessive tissue, blood clot and the oesophagus before the analytical procedure. Clearing and staining for cartilage and bone followed a double-staining procedure which is a standard differential colouring technique for differentiating cartilage from bone and elucidating the arrangement of all supporting structures of the syrinx (Cannels 1988). This technique takes at least four days provided there is no interruption during enzyme steps. Certain steps may take longer on larger syringes and

factors such as fixation quality and preservation affect the outcomes. Syringes were stained with Alcian blue for 24 hrs to differentiate cartilages. This was followed by several washes as follows: the syringes were washed in 95% ETOH for several minutes (until specimen sank), washed in 50% ETOH for several minutes (until specimen sank), then rinsed using distilled water and immersed in an enzyme solution (containing 40 ml aqueous sodium borate; 110 ml distilled water; 2 g trypsin) for 24 hrs to clear membranous parts. The syringes were gently rinsed in tap water.

After the washes, the syringes were stained with Alizarin red solution for 24 hrs which targets the calcium phosphate (bone components). The syringes were rinsed gently in tap water and then dehydrated through a series of KOH/glycerine solution for 6 hrs each. A) 3:1 KOH/glycerine:30 ml of 10% KOH; 270 ml distilled water; 3 ml of 3% hydrogen peroxide (bleaches pigments); 100 ml of glycerine. B) 1:1 KOH/glycerine:30 ml of 10% KOH; 270 ml of distilled water; 3 ml of 3% hydrogen peroxide; 300 ml of glycerine. C) 1:3 KOH/glycerine:15 ml of 10% KOH; 135 ml of distilled water; 450 ml of glycerine. The syringes were stored in a solution of 90% glycerine and 10% distilled water.

Cleared and stained syringes were examined using a Leica DFC 295 stereo microscope and the LAS EZ version 1.7.0 software. All these techniques were carried out by the me in Centro de Ecología Aplicada del Litoral-CONICET laboratory in Argentina under the guidance of Dr Kopuchian. The Department of Economic Development Environment and Tourism (*LEDET*) in Polokwane issued me with the exportation permit (CPM 48349).

2.2.2.3 Histological analysis

For the tissue-sectioning procedure, the syringes were transferred from Karnovsky's fixative into cassettes that were then transferred to 10% neutral buffered formalin and kept over-night to further fix the tissues. The tissue processing into thin microscopic sections was done using a paraffin block following these steps:

Dehydration – the syringes were subjected to a series of increasing concentration of alcohol (50%, 70% 80%, 90%, 95% and 100% for 10 minutes each), and propylene oxide 100% for 20 minutes to remove the water and formalin from the tissue.

Clearing – the syringes were then immersed in xylene three times for 10 minutes each to remove alcohol in the tissue to allow infiltration with paraffin wax.

Embedding – this is the final step before sectioning. The syringes were infiltrated by the paraffin wax which surrounded the whole specimen creating what is known as "block". Once the block solidifies, sectioning becomes easier.

Sectioning – before sectioning, the specimens were chilled on an ice ray for 10 – 15 minutes. A microtome was used to slice sections of 4 µm thickness. Once cut, the tissue ribbons were transferred to a warm water bath held at 45°C where they float on the surface; and can be lifted onto a slide placed underwater.

Paraffin wax removal - The slides were immersed in xylene twice for 10 minutes, followed by ethanol (100%, 100%, 80% and 70% for 5 minutes each) The slides were organised in an upright position and allowed to dry at 37°C for a few hours to gently allow the excess paraffin wax to melt leaving only the sectioned tissue on the slide intact.

Staining - two stains were used, namely the haematoxylin and eosin stain (Bancroft and Gamble 2002) for more general biological examination. Following the stains, a coverslip was mounted over the specimen on the slide, using optical glue to protect the specimen.

2.3 Results

The number of syringes examined for each species per clade (the Alaudid - A, Mirafrad - B and Ammomanid - C) in gross morphology and histology are summarised in Table 2.1 and Table 2.2, respectively.

2.3.1 Syringeal gross morphology

2.3.1.1 Description of study taxa

In this study, King (1989) and Düring ***et al's***. (2013) terminology was followed in labelling the syringeal muscles since they studied passerine birds. The tracheal rings, tympanum and bronchial rings are calcified/mineralised/ossified with a show of red colour from Alizarin red while cartilages stained blue with Alcian blue and muscles were not

specifically stained and thus appear buffish-white, sometimes with a cloud of blue colour from Alcian blue. In this way, the different components were differentiated (Figs. 2.2, 2.3).

Across the three clades (A, B, C), as identified by Alström *et al.* (2013), gross morphologically, the larks were found to possess a typical syrinx classified as '*syrinx tracheo-bronchialis*'. This type of syrinx is composed of the trachea-syringeal cartilage, tympanum, bronchosyringeal cartilage and pessulus (Fig. 2.2). This implies that the syrinx formation encompasses both the tracheal and bronchial tissues and, perhaps these tissues may play a role in shaping the structure of vocalisation of larks.

Figure 2.3 shows the *trachea* (unpaired tube consisting of ossified tracheal rings that join the larynx to the bronchus), a *tympanum* (unpaired, ossified cylinder formed by fused tracheal rings, found at the caudal end of the trachea), *pessulus* (unpaired, ossified cartilage, situated at the caudal end of the tympanum, derived from the fusion of two bronchial half-rings), and bronchus (paired tube consisting of *cartilago bronchialis:* (paired, ossified C-shaped cartilaginous rings starting from B4, B5 to B8, which connect trachea to the lungs).

Five types of muscle and one type of cartilage were identified in the syrinx of larks and they are:

1) *Musculus tracheolateralis* are paired muscles that forms an extended band along the trachea attaching caudally and ventrally to the syrinx.

2) *Musculus sternotrachealis* are paired muscles that attach to the tracheal ring T1 and the bulge of the sternum.

3) *Musculus syringealis ventralis* are paired muscles that attach to the tympanum.

4) *Musculus tracheobronchialis ventralis superficials* are paired muscle that attaches to tracheal rings T4 and T5 along with bronchial half-ring B3.

5) *Musculus tracheobronchialis brevis* are paired muscle that attach to the tympanum and bronchial half-ring B2.

6) *Cartilago trachealis* are unpaired, ossified cartilaginous rings forming the trachea.

There are differences in the gross morphological structure of the syrinx of larks across all the clades. These differences are as reflected in Figure 2.4 and Appendix 2.1. The differences are as follows:

1) the degree of bronchial ring ossification (whether the ossification is restricted to the centre of the bronchial rings with the parts towards the tips of the rings remaining unossified – Fig. 2.4a and b or rings are almost fully ossified – Figs. 2.4c and d);

2) bronchial ring ossification pattern (whether bronchial ossification shows a serial pattern – Fig. 2.4a and b or a non-serial pattern – Figs. 2.4c and d);

3) bronchial ring completeness (almost joined/closed C-bronchial ring – Figs. 2.4c or open C-rings – Figs. 2.4a, b and d);

4) the presence (Figs. 2.4c, d) or absence of the divided or double bronchial rings (Figs. 2.4a and b; and

5) the presence (Fig. 2.4b) or absence (Figs. 2.4a, c and d) of an oblique muscle-like structure (name is unknown) on the ventral side.

Clade A

Galerida magnirostris, Spizocorys conirostris, S. sclateri, and *S. starki* (Appendices 2.A.2 - 2.A.5) have bronchial rings that are almost fully ossified except for a single individual each in *G. magnirostris* (Appendix 2.A.2a) and *Calandrella cinerea* (Appendices 2.A.1a-c) where the ossification was restricted to the centre of the bronchial rings. The pattern of ossification in the bronchial rings of Large-billed Lark *G. magnirostris,* Pink-billed Lark *S.*

conirostris, Sclater's Lark *S. sclateri* and Stark's Lark *S. starki* (Appendices 2.A.2 – 2.A.5) does not follow a serial pattern while serial ossification of the bronchial rings is observed in Red-capped Lark *Calandrella cinerea* (Appendix 2.A.1). The serial ossification pattern means that the degree of ossification on the bronchial rings can either be dominant on the lateral side while the ventral side of the bronchial rings is not fully ossified. The bronchial rings of *G. magnirostris, S. conirostris, S. sclateri* and *S. starki* are almost complete or closed while *Calandrella cinerea* has open bronchial rings (Fig. 2.2). There is also an unusual observation of double or divided bronchial rings in some of the bronchial rings that join each other before completing the turn on the lateral side of the syrinx in three of the four Large-billed Larks and one of the three Red-capped Larks*.* All the examined species lack the oblique muscle-like structure that pierces through on the ventral view of one syrinx only of Spike-heeled Lark *Chersomanes albofasciata*.

Clade B

*A*ll the species belonging to genus *Calendulauda* have ossification of the bronchial rings restricted to the centre, with the serial ossification pattern and have almost open, C-bronchial rings (Appendices 2.B.5 – 2.B.9). In the genus *Mirafra*, the bronchial rings were fully ossified but with non-serial ossification pattern and almost closed, except in Melodious Lark *Mirafra cheniana* (Appendix 2.B.1a), Eastern Clapper Lark *M. fasciolata* (Appendices 2.B.3c and d) and in one of the Monotonous Lark *M. passerina* individuals (Appendix 2.B.4b) where the ossification is restricted to the centre of the bronchial rings with serial ossification pattern and open, C-bronchial rings. In this clade, the presence of double or divided bronchial rings in some bronchial rings that join each other before completing the turn on the lateral side of the syrinx were observed in one individual each of Fawn-coloured Lark *Calendulauda africanoides* (Appendix 2.B.5a) and Sabota Lark *C. sabota* (Appendix 2.B.9a)*.* All the examined members of clade B lack the oblique muscle-like structure on the ventral view of *C. albofasciata*.

Clade C

Among the members of clade C, the bronchial rings that have ossification restricted to the centre were observed in both Karoo Long-billed Lark *Certhilauda subcoronata* specimens

(Appendices 2.C.1a – b) but in only one Cape Long-billed Lark *C. curvirostris* (Appendix 2.C.2a) and two Grey-backed Sparrow-Lark *Eremopterix verticalis* (Appendices 2.C.3a – b) which, unlike other individuals with full bronchial ring ossification, surprisingly have a serial pattern of ossification and open C-bronchial rings. The double or divided bronchial ring was only observed in one of the two Cape Long-billed Lark (Appendix 2.C.2a). Among all the individual syringes examined across the three clades and the selected outgroup species, the presence of an oblique structure that pierces through on the ventral view of the syrinx was observed in one *C. albofasciata* specimen (Appendix 2.C.5b).

2.3.1.2 Description – outgroup taxa

The outgroup species were selected from five genera representing five families. Only one syrinx was examined for each of the species. Among the outgroups, the bronchial rings that were almost fully ossified in a non-serial manner and with almost closed C-bronchial rings were observed in Long-billed Crombec *Sylvietta rufescens*, African Pipit *Anthus cinnamomeus* and Rattling Cisticola *Cisticola cheniana* though with a serial ossification pattern (Appendices 2.O.1, 2.O.4, 2.O.5). On the other hand, Garden Warbler *Sylvia borin* and Lesser Striped Swallow *Hirundo abyssinica* (Appendices 2.O.2, 2.O.3) have ossification restricted to the centre of the bronchial rings with a serial pattern of ossification and open C-bronchial rings. The presence of double or divided bronchial rings was only observed in Lesser Striped Swallow among outgroups. The outgroup taxa were marked by the absence of the obliquely positioned muscle-like structure found on the ventral view of *C. albofasciata*.

2.3.2 Syringeal histology

2.3.2.1 Description – study taxa and outgroups

The syringeal samples analysed for the histology component are shown in Table 2.2. Unlike in the syringeal gross morphological analysis, the sampling representation in histological was unfortunately, sparse across each of the clades (A, B) with clade C (the ammomanid clade) having no representation (Table 2.2). The reason for insufficient sampling was because of the allowed number of specimens to be short and that certain

species such as Barlow's Lark *Calendulauda barlowi* is of conservation concern and cannot be collected.

The histological analyses revealed differences based on the shape of pessulus, its position relative to the bronchial rings 1, 2 and 3 (B1, B2 and B3 respectively), length of the internal tympaniform membranes, as well as connective tissue along the internal tympaniform membrane. The histological structure of the syringes of the examined larks and their relatives is generally similar, with only a few minor differences observed (Fig. 2.5). The pessulus is observed in all the species of the larks (in some cases large e.g. in *G. magnirostris*) and the outgroup species sampled, except in few cases where it was difficult to observe the structures when parts of the tissues were damaged. The pessulus is well develop, sometimes large but showing no ossification. The pessulus varies in shape and size. *Galerida magnirostris* has a blunt-shaped pessulus which is large (Appendix 2.5.1, 127c). In Eastern Clapper Lark *M. fasciolata* (Appendix 2.5.2, 117d), Monotonous Lark *M. passerina* (Appendix 2.5.4, 132a) and Melodious Lark *M. cheniana* (Appendix 2.5.3, 123a), the pessulus is pointy or sharp while it was observed to be blunt in another syrinx of Melodious Lark (Appendix 2.5.3, 116c). Among the outgroups, the blunt pessulus is observed in African Pipit *Anthus cinnamomeus* (Appendix 2.5.7, 131b), Plain-backed Pipit *Anthus leucophrys* (Appendix 2.5.6, 29d) and Cape Longclaw *Macronyx capensis* (Appendix 2.5.8, 119b); and it is pointy in Marsh Warbler *Acrocephalus palustris* (Appendix 2.5.5, 143b) and Rattling Cisticola *Cisticola chiniana* (Appendix 2.5.9, 144c).

Among the species that were studied, the pessulus was also found to differ in its position relative to bronchial ring 2 (B2), i.e. whether the pessulus is positioned below B2, above B2 or aligned with B2. Among larks, Large-billed Lark, Monotonous Lark, and one syrinx (Appendices 2.5.1, 2.5.4, 2.5.3:123, respectively) of Melodious Lark the pessulus is positioned above B2. In Eastern Clapper Lark and another Melodious Lark, syringes have a pessulus that aligns to B2 (Appendices 2.5.2, 2.5.3: 116, respectively). Regarding the outgroups, one syrinx of Marsh Warbler (Appendix 2.5.5: 142) had a pessulus positioned beyond B2, while the pessulus was positioned below B2 in Plain-backed Pipit (Appendix 2.5.6). In another syrinx (Appendices 2.5.5, 2.5.7, 2.5.8 and 2.5.9 respectively)

of Marsh Warbler, African Pipit, Cape Longclaw, and Rattling Cisticola, the pessulus aligns with B2.

A typical connective tissue, which is bound by the internal tympaniform membrane and runs along the internal tympaniform membrane, is prominent in Large-billed Lark, Melodious Lark, Eastern Clapper Lark, Cape Longclaw. This connective tissue is restricted towards the pessulus in Monotonous Lark, Plain-backed Pipit, Marsh Warbler, African Pipit and Rattling Cisticola. The internal tympaniform membrane differs in length from the pessulus towards the medial bronchial wall. Large-billed Lark, Eastern Clapper Lark, one syrinx of Melodious Lark (Appendix 2.5.3, 116) and the outgroup species African Pipit had a short internal tympaniform membrane while it was considerably long in one syrinx of Melodious Lark (Appendix 2.5.3, 123), Monotonous Lark, and the outgroups Plain-backed Pipit, Marsh Warbler, Cape Longclaw and Rattling Cisticola.

2.4 Discussion and Conclusions

Although not all the species representing the different lark clades were examined and despite that there is uncertainty as to the closest evolutionary relatives of larks, the findings presented in this chapter have shed some light. This is concerning the general morphology and histological structures of the syringes of larks and the species deemed to be the putative relatives of larks. Like several other passerine species and families, larks possess a '*syrinx tracheo-bronchialis*' (Suthers *et al.* 1999, Tsukahara *et al.* 2008, Düring *et al.* 2013, García *et al.* 2017, Christensen *et al.* 2017) with both the tracheal and bronchial tissues taking part in its formation. This type of syrinx is also prevalent among non-passerines, e.g. Galliformes and Anseriformes.

At a gross morphological level, similar findings were recorded from multiple syringes from individual birds belonging to the same species with some intraspecific variation in some species. Intraspecific variation is with regard to the presence or absence of divided bronchial rings, the degree and pattern of ossification, and/or the presence or absence of the oblique muscle-like structure. Unfortunately, sample sizes for some species were too small to establish the extent of intraspecific variation with regard to histological features. There are syringeal differences across the three clades as well as between the larks and the outgroups selected in this study which allowed comparison of

the syringeal structures of larks with those of the outgroups. However, with all the differences and similarities in the structure of the syringes found between individuals and species and also across the clades, it is difficult to determine whether all these could be attributed to sex of individual birds due to unavailability of sex information for most species.

In terms of the gross morphology of the syrinx, it is apparent that some characters were found to exist in clades to varying degrees; some were found to be genus-, species- and individual-specific. The double or divided bronchial ring was present in all the three clades (A-Alaudid, B-Mirafrid and C-Ammomanid) but only in certain genera, species and individuals within a clade. One outgroup species, *Hirundo abyssinica* also had a divided bronchial ring and this was also reported in the family Hirundinidae in Ames (1971). The divided or double bronchial ring is a character that has also been observed for example in clade A: *G. magnirostris*, *C. cinerea*; clade B: *C. africanoides*, *C. sabota* and clade C: *C. curvirostris*. The oblique muscle-like structure on the ventral side of the syrinx was absent in clade A, B and the outgroups, but present in clade C in one individual syrinx of *C. albofasciata* (Appendix 2.3.5 b). The fact that this structure was only found in a syrinx of a single individual bird makes it is difficult to draw conclusions about its prevalence and function as none of the literature consulted in this study reported its existence. What is evident is that the syringes of adult birds across the three clades and the outgroup species have bronchial rings that are either almost closed forming C-rings, fully ossified with the ossification forming a serial pattern, or open C-bronchial rings and with ossification that is restricted to the centre of the bronchial rings and have a serial ossification pattern.

The small sample sizes in this study highlighted the need for more sampling to include more species and genera, but also more individuals per species to adequately account for intraspecific variation as was observed in this study. Inadequate sampling may probably have been one of the reasons which led to the confusion pertaining to the notion of either the presence or absence of a pessulus in Alaudidae. The confusion was exacerbated by the lack of presented syringeal structures in the sources that cited either the ‘absence’ or ‘presence’ of pessulus in the syrinx of larks (Ames 1971). This study confirms the presence of a pessulus in the syrinx of larks which appears large in some individual birds of some species e.g. *G. magnirostris*. In de Juana *et al.* (2004), the

pessulus was considered rudimentary. Personal communication between de Juana and my co-supervisor Dr Kopuchian, revealed that the information provided was obtained from Verheyen (1958). However, Verheyen's study only mentioned larks having a rudimentary pessulus but neither information about the source of this view was given in the manuscript nor were the diagrams of the syringes of larks showing the pessulus shown. Therefore, to this end, the present study refutes the notion that larks do not possess a pessulus as stated in the work of Mayr and Amadon (1951), Ames (1971) and also King and McLelland (1984). The findings are however, consistent with de Juana *et al.* (2004) and Dean and Williams (2004) about the presence of a pessulus regardless of the lack of diagrams or any evidence to show this feature and the rudimentary state. Further to this, this study is not in a position to determine whether the pessulus is ossified or not since the ossification stain was not used in the histological analysis. In gross morphological analysis of the syringes, the bone stain was used but due to the large quantities of muscles covering the syringes ossification was only observed in one specimen which was not wholly covered with muscles

Histologically, there are differences across the clades and among the outgroup species. Despite the limited sampling and small sample size, the findings of this study provide some answers regarding the structure of the syrinx of larks. Undoubtedly, gross morphological and histological analyses of the syrinx complement each other, particularly in the Alaudidae. For example, in gross morphology, the large quantity of the *Musculus syringealis ventralis* on the ventral view of the syrinx obstruct a good view of the pessulus and it was through the histological analysis that the presence of pessulus was confirmed.

To conclude the syringeal gross morphology aspect of this study, the results demonstrated that some characters exist in clades to varying degrees, some were found to be genus-, species- and individual-specific. For example, at genus level, there is consistent full ossification in all sampled genera in clade A except in the genus *Calandrella*. In clade B the two sampled genera are different in terms of pattern of bronchial ossification with *Mirafra* showing non-serial ossification as opposed to the serial ossification of members of the genus *Calendulauda*. In the case of intraspecific differences in gross morphology of the syrinx, double or divided bronchial rings was a character that differed from one individual to another, e.g. in *Galerida magnirostris* one

individual didn’t have divided bronchial rings, and also the presence of the muscle-like oblique structure in one *Chersomanes albofasciata* individual but not the others.

An aspect that this study did not focus on was the functional significance of each of the parts of the syrinx in voice production. This study provided a baseline about the structure of the syrinx of larks and also removes ‘confusion’ in literature with regard to the presence of pessulus. There is no doubt that the study has revealed characters already known to exist in other avian taxa and those that were not yet recorded for larks in literature or uncommon in avian taxa in general. However, there is a need for further analyses of multiple syringes of the other lark species not presented in this study so that a more complete picture can be obtained given that the syrinx is of taxonomic importance among passerines. Both gross morphology and histological perspectives will be critical.

TABLE 2.1. LIST OF THE LARK AND OUTGROUP SPECIES INCLUDED IN THE GROSS MORPHOLOGICAL ANALYSIS OF THE SYRINGES EXAMINED.

No.	Scientific name	English name	Degree of bronchial ossification	Bronchial ossification pattern	Bronchial ring completeness	Double/divided bronchial rings	Oblique muscle-like structure
			almost to full ossification	non-graded	almost closed (C-ring)	absent	absent
			restricted to the centre of bronchial rings	graded series	Open (C-ring)	present	present
	CLADE A - Alaudid						
42	*Galerida magnirostris*	Large-billed Lark	almost to full ossification	non-graded	almost closed	present	absent
43	*Galerida magnirostris*	Large-billed Lark	almost to full ossification	non-graded	almost closed	present	absent
77	*Galerida magnirostris*	Large-billed Lark	almost to full ossification	non-graded	almost closed	absent	absent
80	*Galerida magnirostris*	Large-billed Lark	almost to full ossification	non-graded	almost closed	present	absent
60	*Spizocorys conirostris*	Pink-billed Lark	almost to full ossification	non-graded	almost closed	absent	absent
61	*Spizocorys conirostris*	Pink-billed Lark	almost to full ossification	non-graded	almost closed	absent	absent
73	*Spizocorys sclateri*	Sclater's Lark	almost to full ossification	non-graded	almost closed	absent	absent
74	*Spizocorys sclateri*	Sclater's Lark	almost to full ossification	non-graded	almost closed	absent	absent
75	*Spizocorys sclateri*	Sclater's Lark	almost to full ossification	non-graded	almost closed	absent	absent
91	*Spizocorys starki*	Stark's Lark	almost to full ossification	non-graded	almost closed	absent	absent
62	*Calandrella cinerea*	Red-capped Lark	restricted to the centre of bronchial rings	graded series	open	present	absent

63	*Calandrella cinerea*	Red-capped Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
64	*Calandrella cinerea*	Red-capped Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
	CLADE B - Mirafrid						
76	*Calendulauda africanoides*	Fawn-coloured Lark	restricted to the centre of bronchial rings	graded series	open	present	absent
99	*Calendulauda africanoides*	Fawn-coloured Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
86	*Calendulauda albescens*	Karro Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
100	*Calendulauda albescens*	Karro Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
87	*Calendulauda burra*	Red Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
88	*Calendulauda burra*	Red Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
89	*Calendulauda burra*	Red Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
96	*Calendulauda erythrochlamys*	Dune Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
92	*Calendulauda sabota*	Sabota Lark	restricted to the centre of bronchial rings	graded series	open	present	absent
94	*Calendulauda sabota*	Sabota Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
95	*Calendulauda sabota*	Sabota Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
97	*Calendulauda sabota*	Sabota Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
72	*Mirafra cheniana*	Melodious Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
45	*Mirafra africana*	Rufous-naped Lark	almost to full ossification	non-graded	almost closed	absent	absent
52	*Mirafra africana*	Rufous-naped Lark	almost to full ossification	non-graded	almost closed	absent	absent
58	*Mirafra africana*	Rufous-naped Lark	almost to full ossification	non-graded	almost closed	absent	absent
44	*Mirafra fasciolata*	Eastern Clapper Lark	almost to full ossification	non-graded	almost closed	absent	absent

50	*Mirafra fasciolata*	Eastern Clapper Lark	almost to full ossification	non-graded	almost closed	absent	absent
51	*Mirafra fasciolata*	Eastern Clapper Lark	almost to full ossification	non-graded	almost closed	absent	absent
56	*Mirafra fasciolata*	Eastern Clapper Lark	almost to full ossification	non-graded	almost closed	absent	absent
136	*Mirafra passerina*	Monotonous Lark	almost to full ossification	non-graded	almost closed	absent	absent
139	*Mirafra passerina*	Monotonous Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
140	*Mirafra passerina*	Monotonous Lark	almost to full ossification	non-graded	almost closed	absent	absent
	CLADE C - Ammomanid						
41	*Certhilauda subcoronata*	Karoo long-billed Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
93	*Certhilauda subcoronata*	Karro long-billed Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
78	*Certhilauda curvirostris*	Cape long-billed Lark	almost to full ossification	non-graded	almost closed	present	absent
79	*Certhilauda curvirostris*	Cape long-billed Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
85	*Eremopterix verticalis*	Grey-backed sparrow-Lark	almost to full ossification	graded series	open	absent	absent
90	*Eremopterix verticals*	Grey-backed sparrow-Lark	almost to full ossification	graded series	open	absent	absent
98	*Eremopterix verticalis*	Grey-backed sparrow-Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
66	*Eremopterix leucotis*	Chestnut-backed sparrow-Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
68	*Eremopterix leucotis*	Chestnut-backed sparrow-Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
69	*Eremopterix leucotis*	Chestnut-backed sparrow-Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent

48	*Chersomanes albofasciata*	Spike-heeled Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
49	*Chersomanes albofasciata*	Spike-heeled Lark	restricted to the centre of bronchial rings	graded series	open	absent	present
53	*Chersomanes albofasciata*	Spike-heeled Lark	restricted to the centre of bronchial rings	graded series	open	absent	absent
	OUTGROUPS						
147	*Sylvietta rufescens*	Long-billed Crombec	almost to full ossification	non-graded	almost closed	absent	absent
145	*Sylvia borin*	Garden Warbler	restricted to the centre of bronchial rings	graded series	open	absent	absent
148	*Hirundo abyssinica*	Lesser-striped Swallow	restricted to the centre of bronchial rings	graded series	open	present	absent
146	*Cisticola chiniana*	Rattling Cisticola	almost to full ossification	graded series	open	absent	absent
124	*Anthus cinnamomeus*	African Pipit	almost to full ossification	non-graded	almost closed	absent	absent

TABLE 2.2. LIST OF THE LARK AND OUTGROUP SPECIES USED FOR HISTOLOGICAL ANALYSIS OF THE SYRINGES EXAMINED.

No.	Scientific name	English name	Pessulus shape	Pessulus position relative to bronchial ring 2 (B2)	Internal tympaniform membrane length	Connective tissue along internal tympaniform membrane
			sharp/pointy	beyond B2	short	restricted towards the pessulus
			blunt	align with B2	long	down along the internal tympaniform membrane
	CLADE A - Alaudid					
127	*Galerida magnirostris*	Large-billed Lark	blunt	beyond B2	short	down along the internal tympaniform membrane
113	*Galerida magnirostris*	Large-billed Lark	blunt	beyond B2	short	down along the internal tympaniform membrane
	CLADE B - Mirafrid					
117	*Mirafra fasciolata*	Eastern Clapper Lark	sharp	align with B2	short	down along the internal tympaniform membrane
123	*Mirafra cheniana*	Melodious Lark	sharp	beyond B2	long	down along the internal tympaniform membrane
116	*Mirafra cheniana*	Melodious Lark	blunt	align with B2	short	down along the internal tympaniform membrane
132	*Mirafra passerina*	Monotonous Lark	sharp	beyond B2	long	restricted towards the pessulus
	OUTGROUPS					
143	*Acrocephalus palustris*	Marsh Warbler	sharp	align with B2	long	down along the internal tympaniform membrane
144	*Cisticola chiniana*	Rattling Cisticola	sharp	align with B2	long	down along the internal tympaniform membrane
131	*Anthus cinnamomeus*	African Pipit	blunt	align with B2	short	down along the internal tympaniform membrane
129	*Anthus leucophrys*	Plain-backed Pipit	blunt	beyond B2	long	restricted towards the pessulus
119	*Macrorynx capensis*	Cape Long-claw	blunt	align with B2	long	down along the internal tympaniform membrane

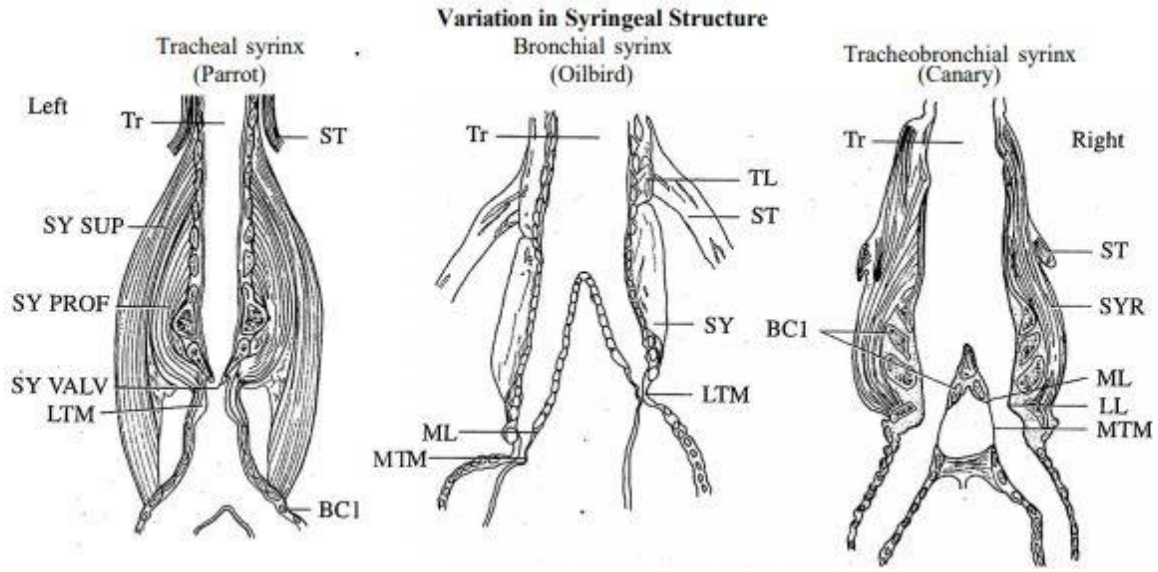


FIGURE 2.1. EXAMPLES OF VARIATION IN SYRINGEAL ANATOMY. THE TRACHEAL PARROT SYRINX HAS TWO SYRINGEAL MUSCLES AND A PAIR OF LATERAL TYMPANIFORM MEMBRANES. THE BRONCHIAL SYRINX OF THE OILBIRD HAS ONE PAIR OF SYRINGEAL MUSCLES AND A PAIR OF MEDIAL AND LATERAL TYMPANIFORM MEMBRANES IN EACH BRONCHUS. SONGBIRDS HAVE SEVERAL PAIRS OF SYRINGEAL MUSCLES IN THEIR TRACHEOBRONCHIAL SYRINX. TR, TRACHEA; ST, STERNOTRACHEALIS MUSCLE; SY SUP, SUPERFICIAL SYRINGEAL MUSCLE; SY PROF, DEEP SYRINGEAL MUSCLE; SY VALV, PNEUMATIC VALVE; LTM, LATERAL TYMPANIFORM MEMBRANE; MTM, MEDIAL TYMPANIFORM MEMBRANE; BCI, FIRST BRONCHIAL CARTILAGE; SY, SYRINGEAL MUSCLE; ML, MEDIAL LABIUM; LL, LATERAL LABIUM; SYR, MUSCLES OF SYRINX. (OILBIRD MODIFIED AFTER SUTHERS AND HECTOR 1985; PARROT AND CANARY MODIFIED AFTER KING 1989).

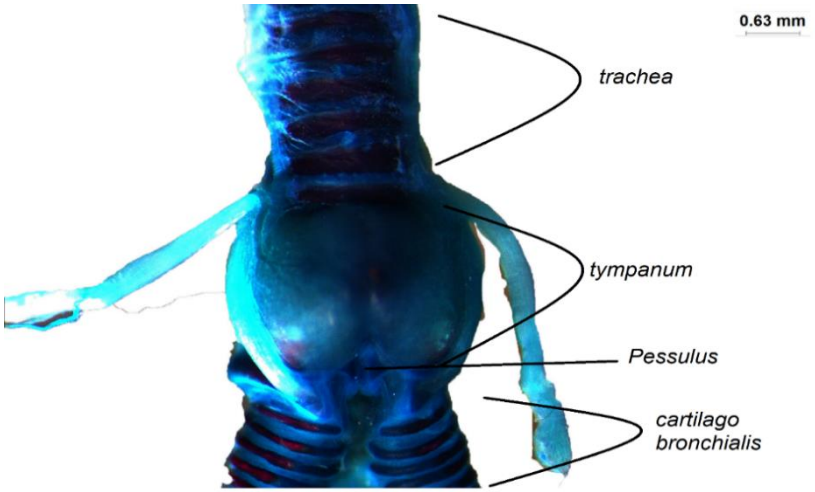


FIGURE 2.2. EXAMPLE OF A VENTRAL VIEW OF THE SYRINX OF A RED-CAPPED LARK *CALANDRELLA CINEREA* ILLUSTRATING THE *SYRINX TRACHEO-BRONCHIALIS* FOUND IN LARKS. THE TERMINOLOGY WAS ADOPTED FROM DÜRING *ET AL.* (2013).

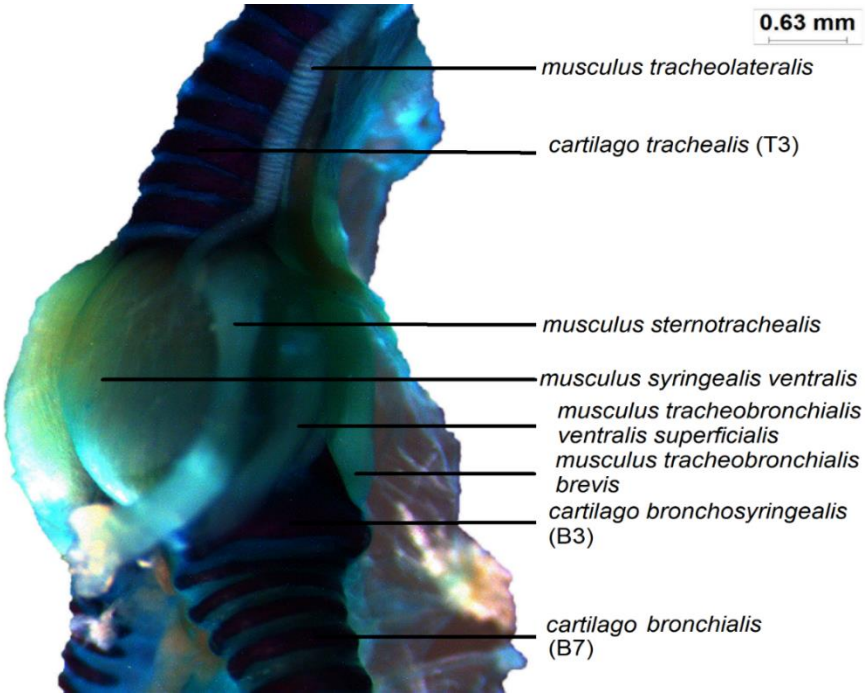
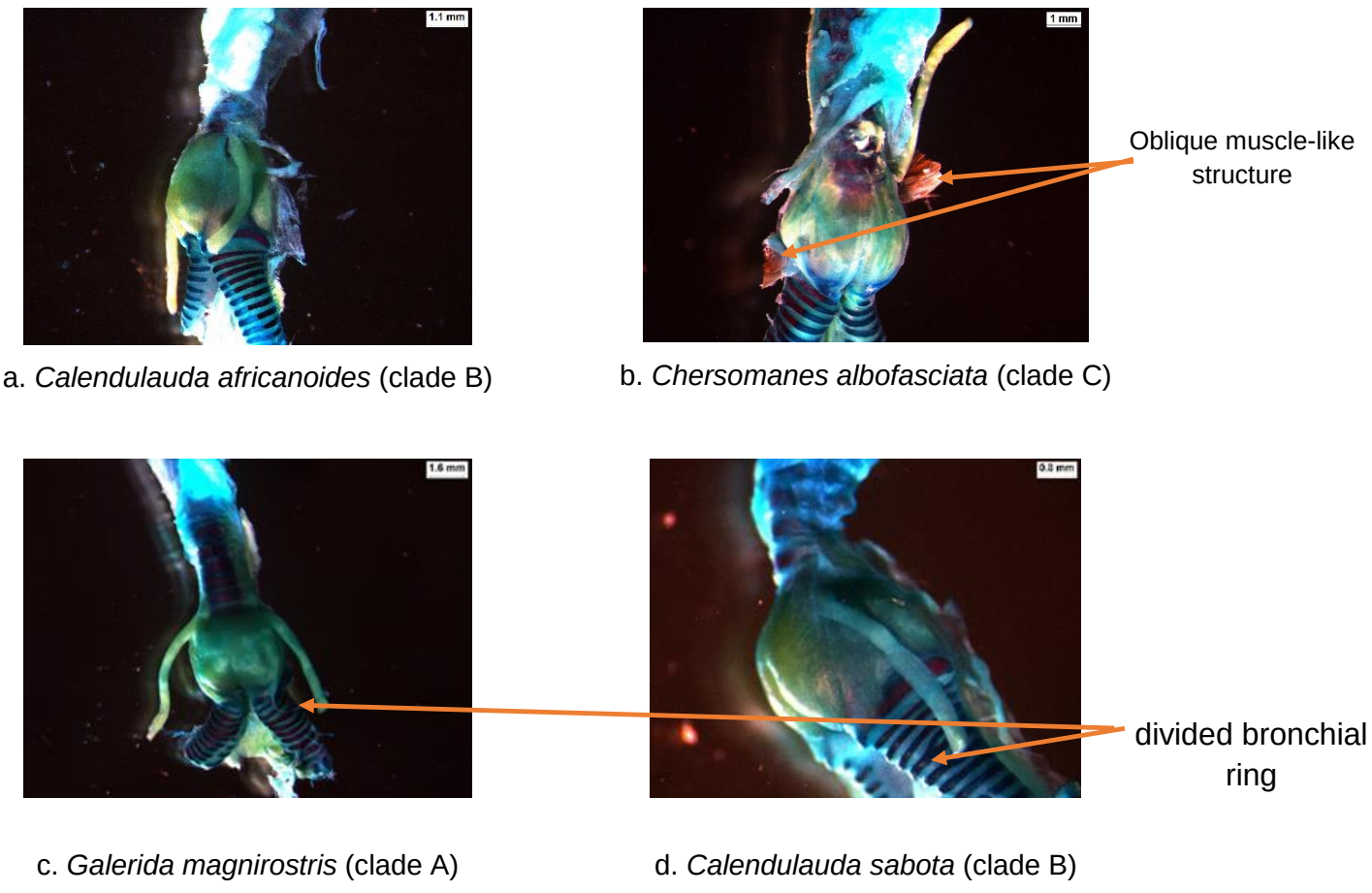


FIGURE 2.3. EXAMPLE OF A VENTRAL VIEW OF THE SYRINX OF A PINK-BILLED LARK *SPIZOCORYS CONIROSTRIS* SHOWING DIFFERENT MUSCLES AND CARTILAGES FOUND IN LARKS. TERMINOLOGY WAS ADOPTED BY DÜRING *ET AL.* (2013).



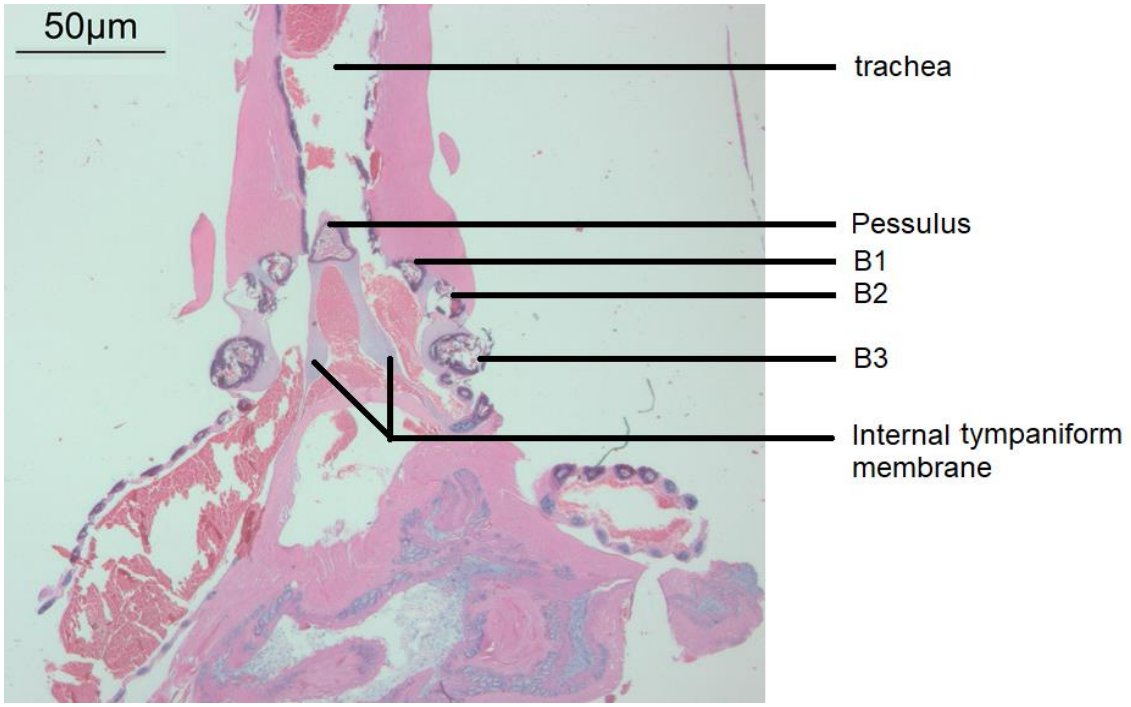
a. *Calendulauda africanoides* (clade B)

b. *Chersomanes albofasciata* (clade C)

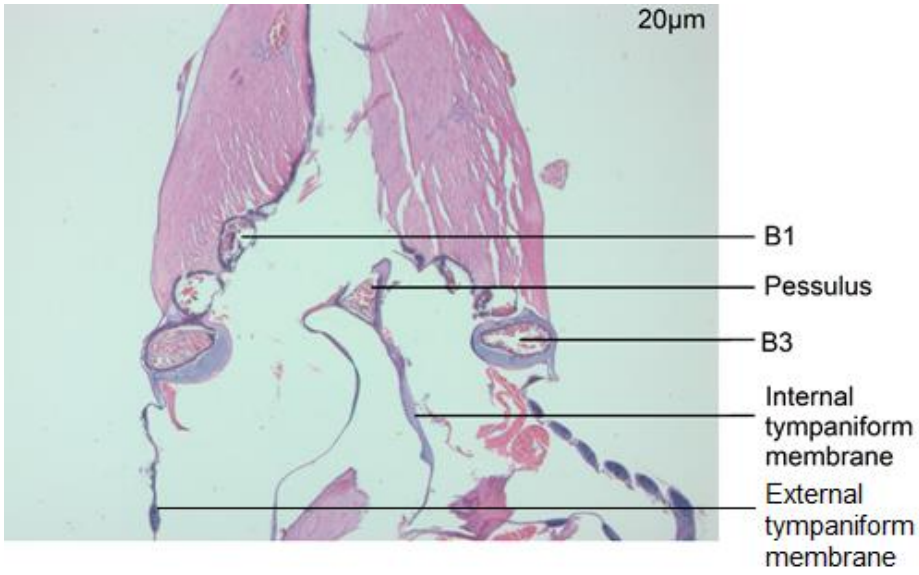
c. *Galerida magnirostris* (clade A)

d. *Calendulauda sabota* (clade B)

FIGURE 2.4. THE THREE MAJOR DIFFERENCES IN THE GROSS MORPHOLOGICAL STRUCTURE OF THE SELECTED SYRINGES OF LARKS ACROSS ALL THE CLADES: A. THE DEGREE OF BRONCHIAL RING OSSIFICATION (FIGS. 2.4A AND B HAVE RESTRICTED OSSIFICATION WHILE 2.4C AND D ARE ALMOST FULLY OSSIFIED), B. BRONCHIAL RING OSSIFICATION PATTERN (SERIAL PATTERN IN FIGS. 2.4A AND B AND NON-SERIAL PATTERN IN FIGS. 2.4C AND D) AND C. BRONCHIAL RING COMPLETENESS (ALMOST JOINED/CLOSED C-BRONCHIAL RING – FIGS. 2.4C OR OPEN C-BRONCHIAL RINGS – FIGS. 2.4A, B AND D).


50µm
trachea
Pessulus
B1
B2
B3
Internal tympaniform membrane

a.



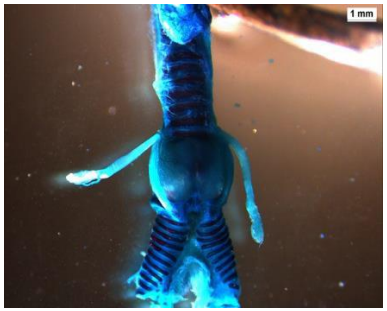
b.

FIGURE 2.5. THE HEMATOXYLIN AND EOSIN STAINED HISTOLOGICAL SECTION OF A TYPICAL LARK (A. LARGE-BILLED LARK *GALERIDA MAGNIROSTRIS*) SHOWING THE SYRINGEAL TISSUE STRUCTURES USED TO COMPARE DIFFERENT SPECIES AND THE OUTGROUPS (B. RATTLING CISTICOLA *CISTICOLA CHINIANA*) USED IN THE STUDY.

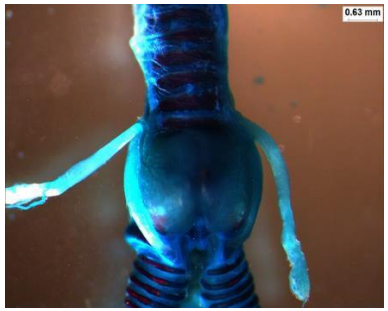
APPENDIX 2.1. GROSS MORPHOLOGY PLATES FOR CLADE A. FOR LABELLING, E.G. APPENDIX 2.A.1 A-C), 2 REFERS TO APPENDIX NUMBER; **A** – CLADE; 1 – SPECIES *CALANDRELLA CINEREA*; NUMBER 62A – SERIES OF PICTURES OF THE SAME SYRINX OF AN INDIVIDUAL BIRD; 63B – SERIES OF PICTURES OF THE SAME SYRINX OF ANOTHER INDIVIDUAL. NB. RED COLOUR – SHOWS OSSIFIED CARTILAGE, BLUE – UNOSSIFIED CARTILAGE, LIGHT BROWNISH COLOUR – MUSCLE.

Calandrella cinerea (Appendix 2.A.1 a-c)

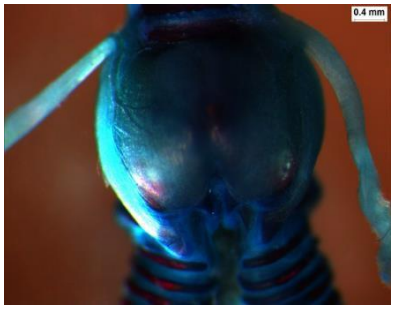
62a



62



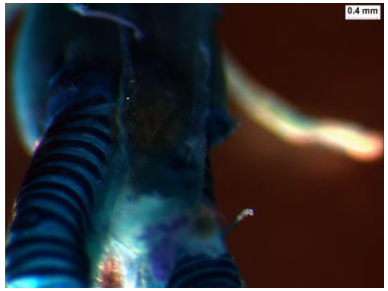
62



62

62

62



62

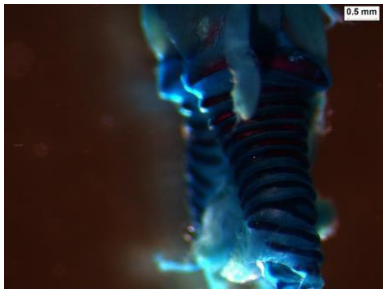
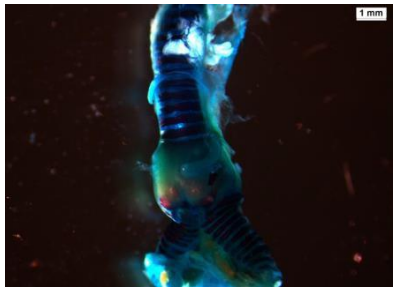
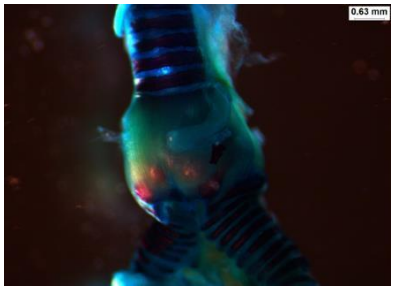
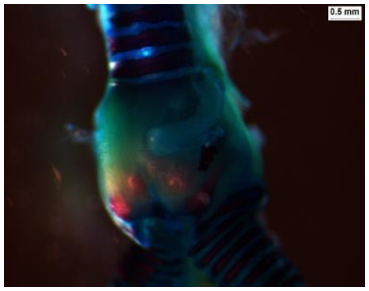
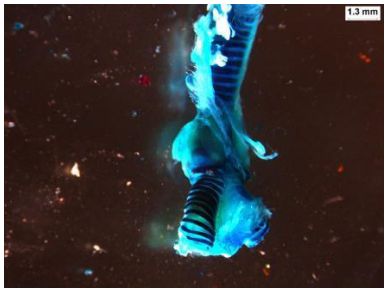
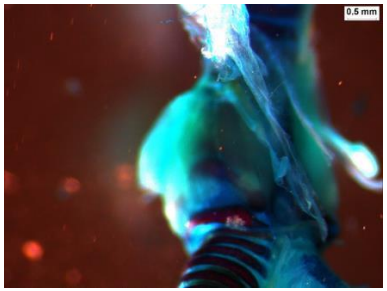
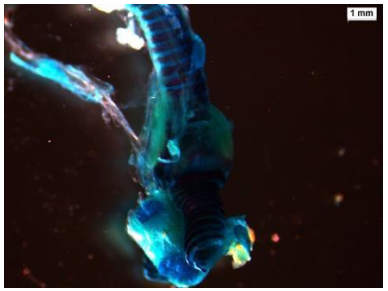
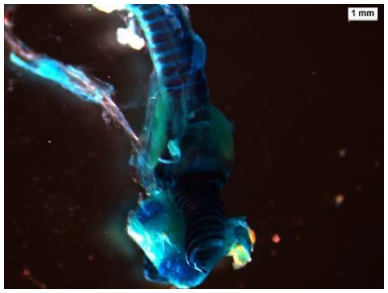
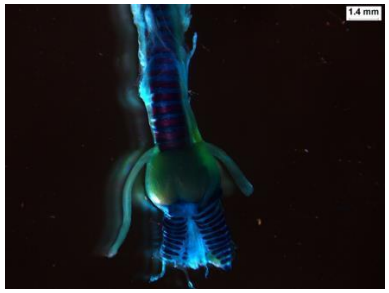
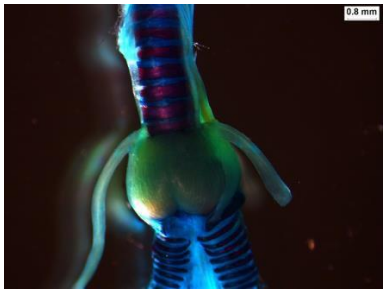
62

62

63b

63

63

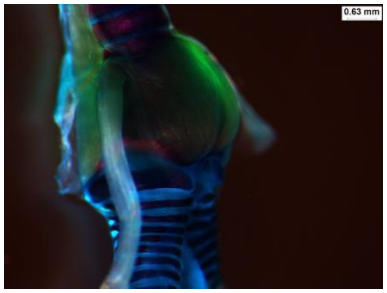
				
Calandrella cinerea	63	63	63	63
				
	63	64c	64	64
				

Galerida magnirostris (Appendix 2.A.2 a-d)

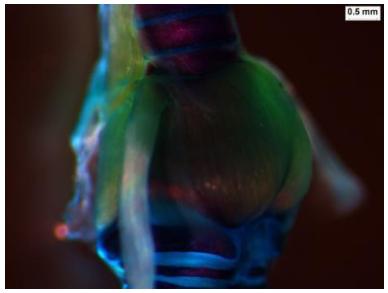
64

64

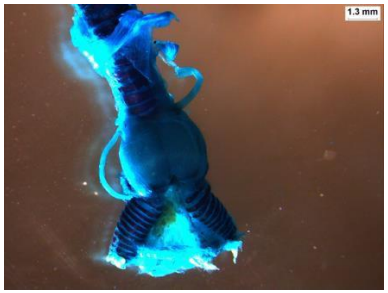
64



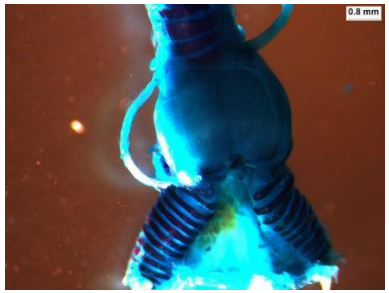
64



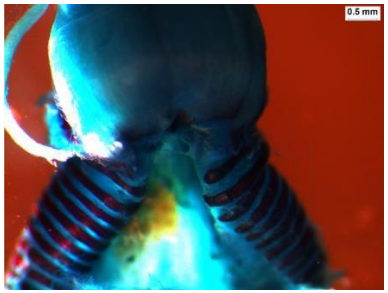
42a



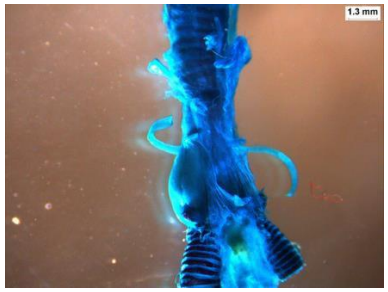
42



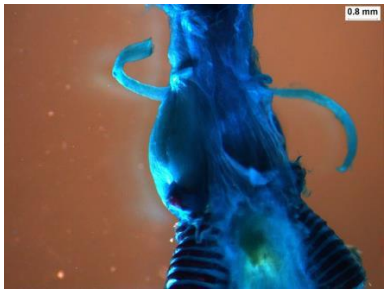
42



42

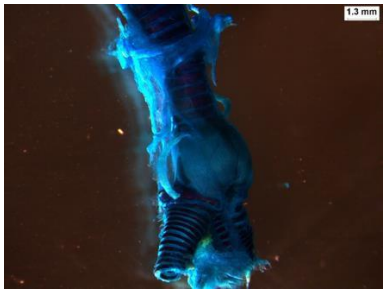


42

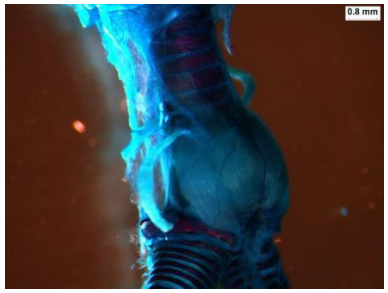


42

42

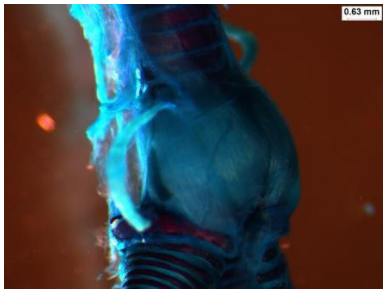


42

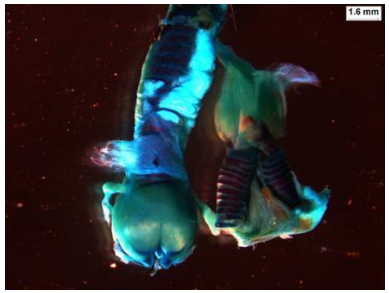


Galerida magnirostris

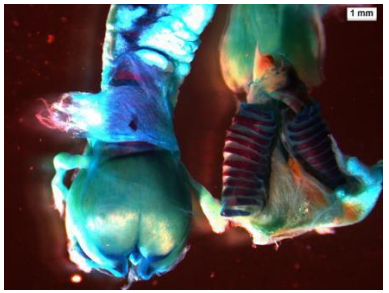
42



43b

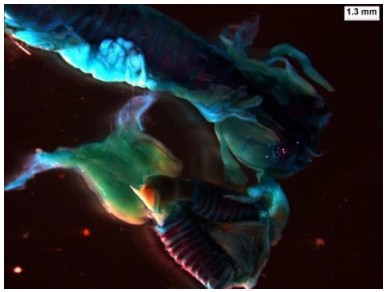


43

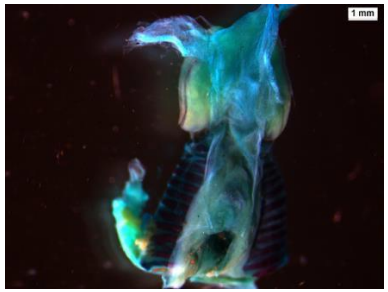


43

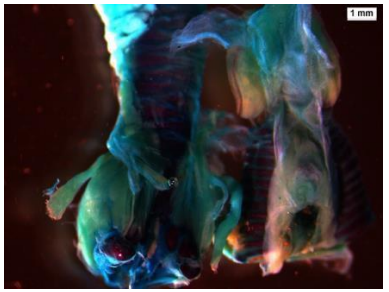
43



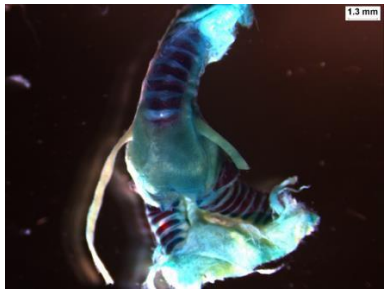
43



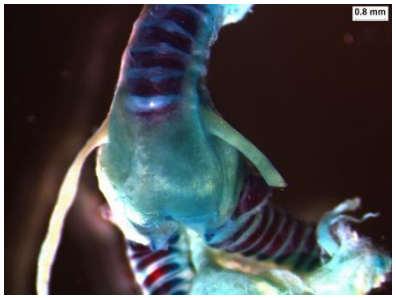
43



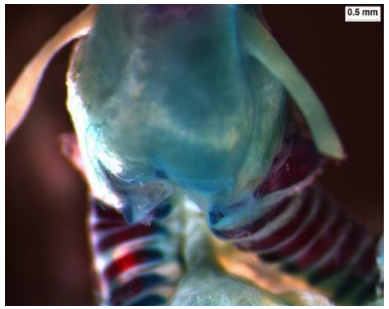
77c



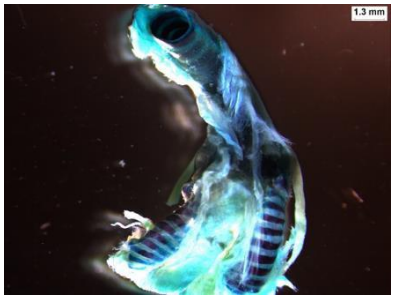
77



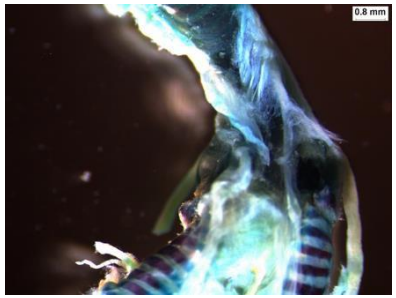
77



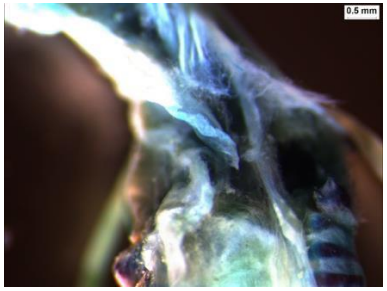
77

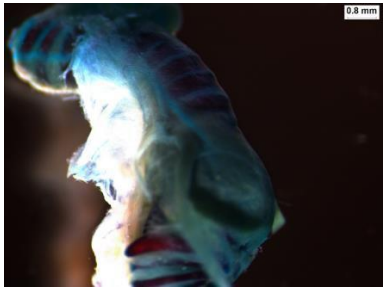


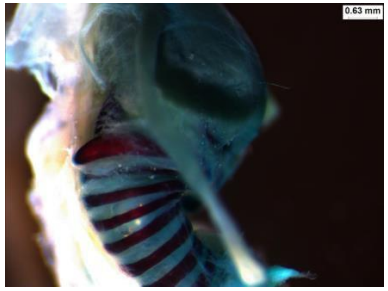
77



77 77 77 77

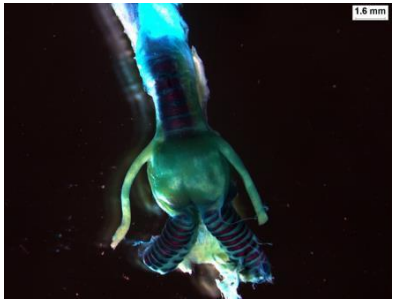


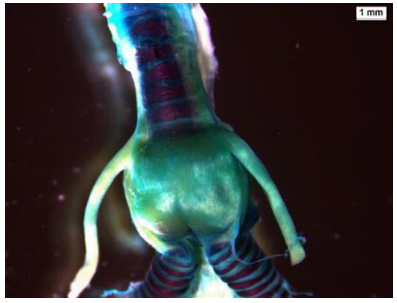


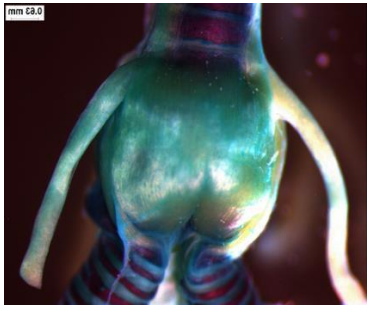
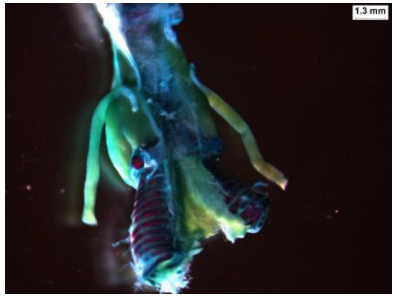


Galerida magnirostris

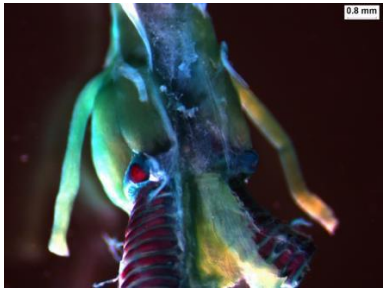
80d 80 80 80

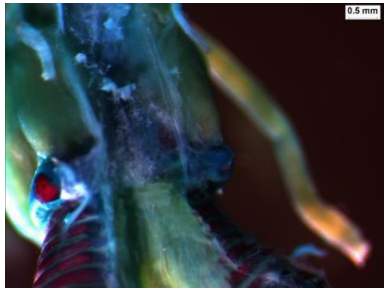


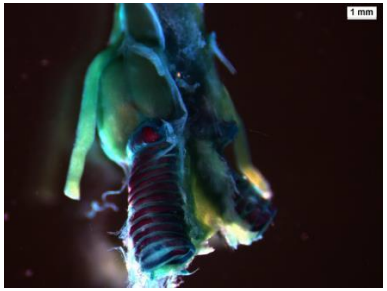


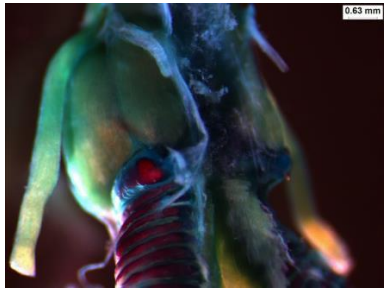



80 80 80 80



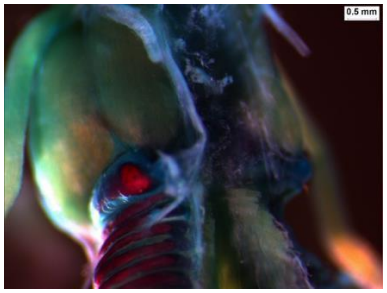




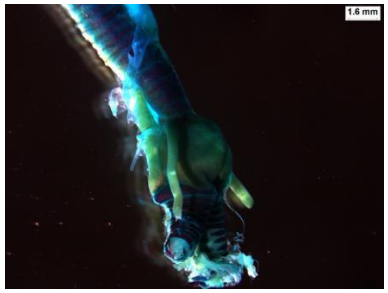


Spizocorys conirostris (Appendix 2.A.3 a-b)

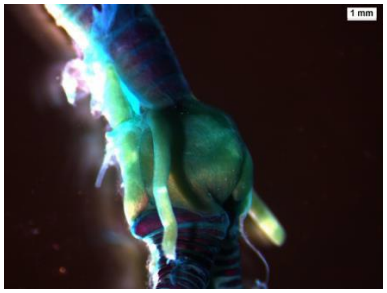
80



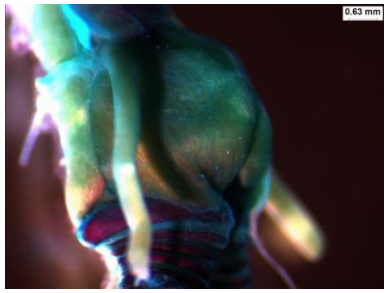
80



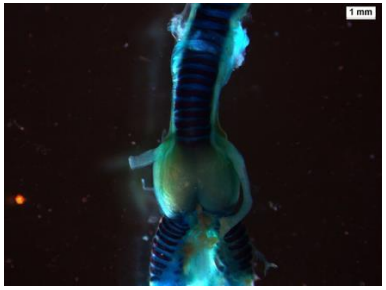
80



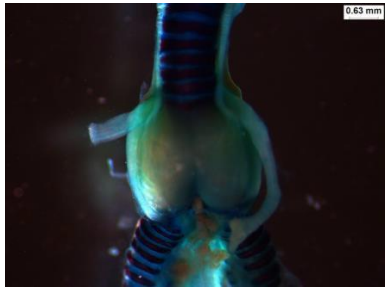
80



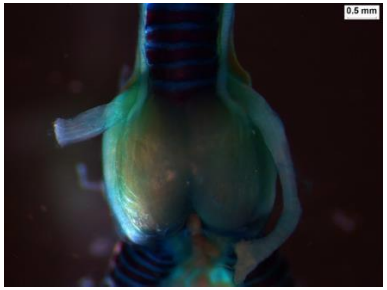
60a



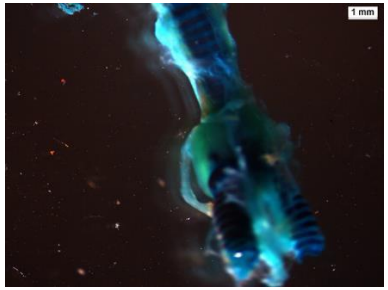
60



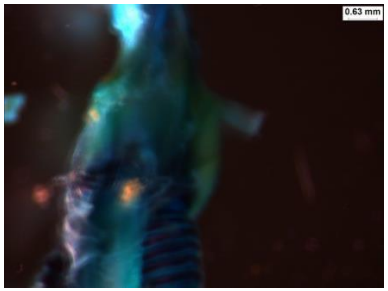
60



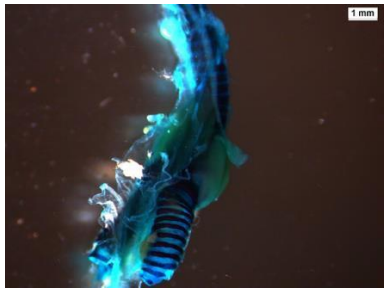
60



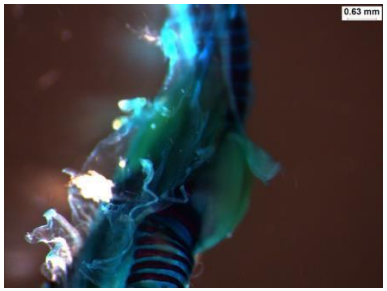
60



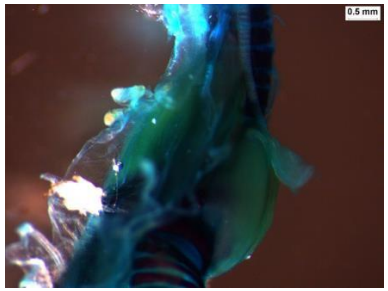
60



60



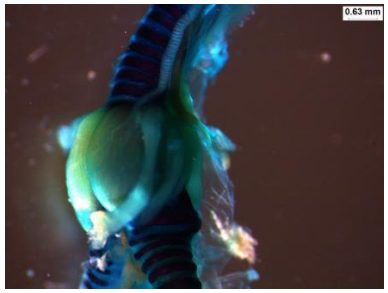
60



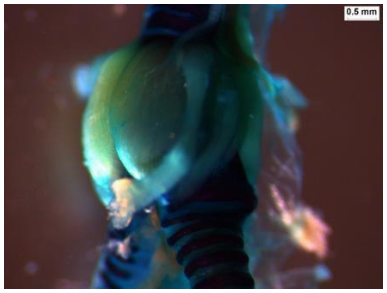
Spizocorys conirostris

60

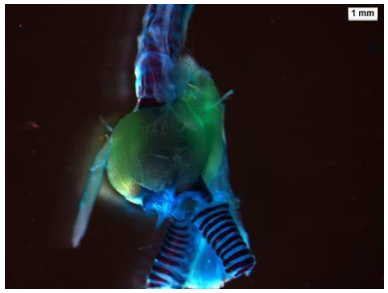
60



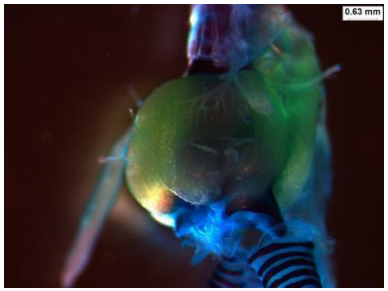
60



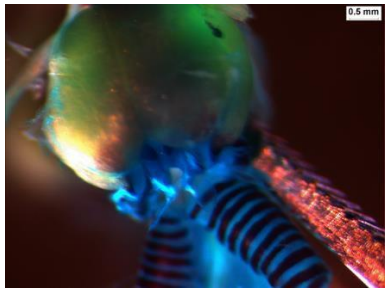
61b



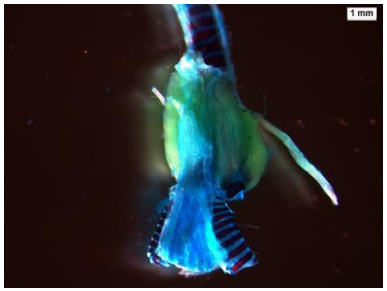
61



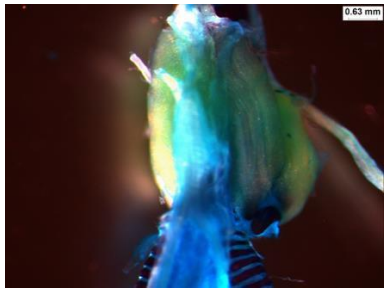
61



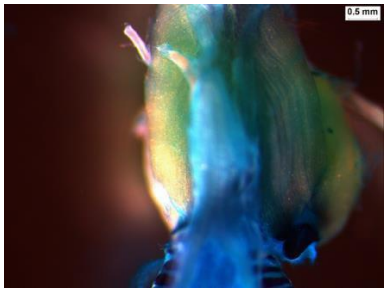
61



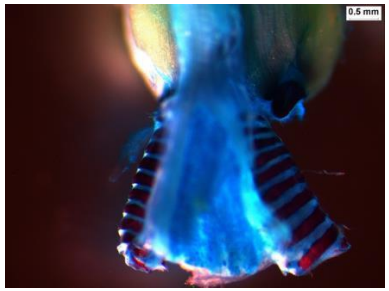
61



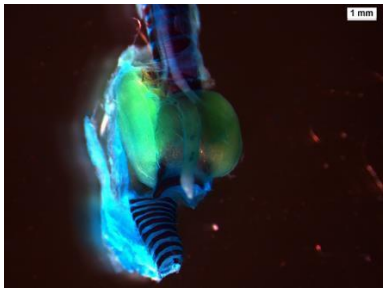
61



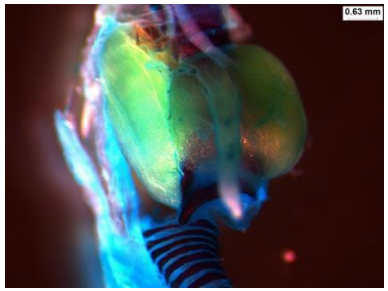
61



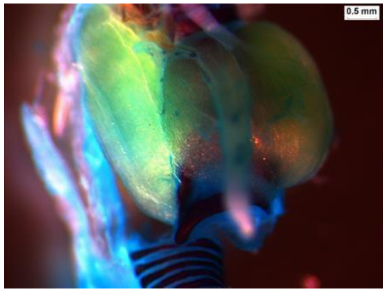
61



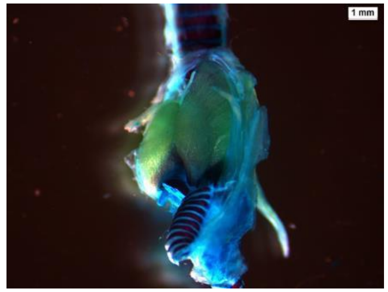
61



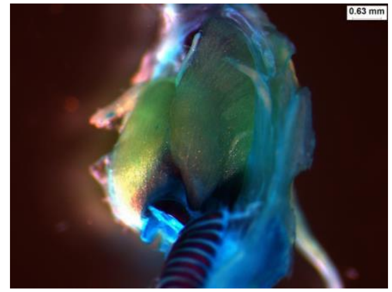
61



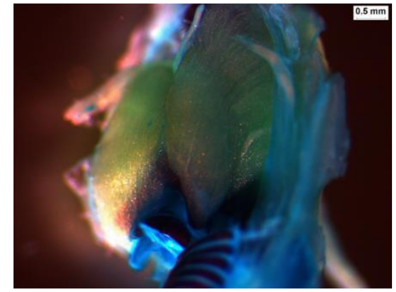
61



61



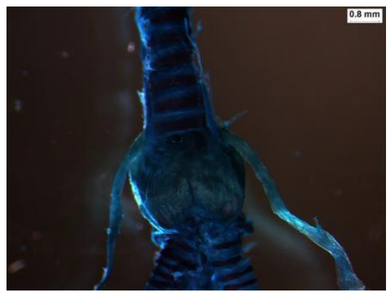
61



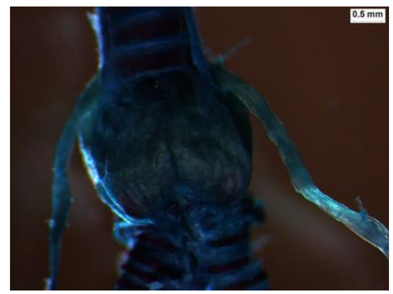
Spizocorys sclateri (Appendix 2.A.4 a-c)

73a

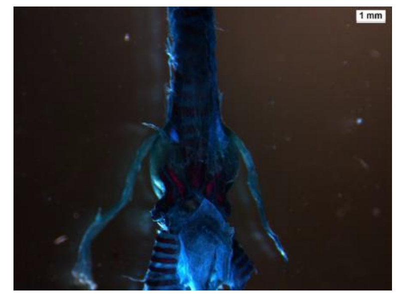
73



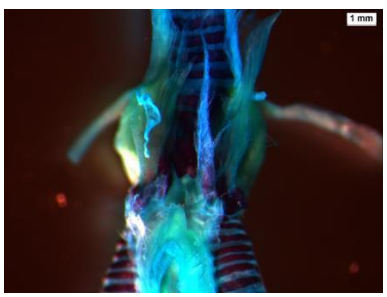
73



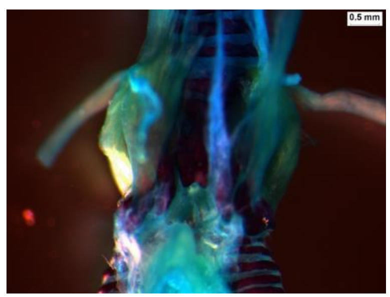
73



73

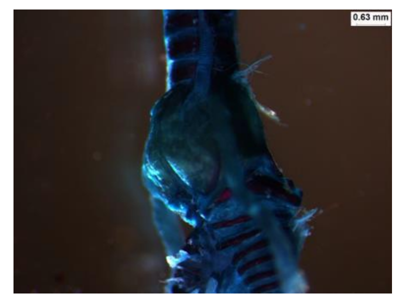


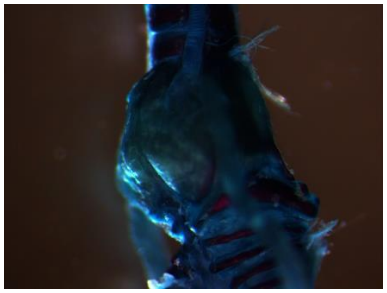
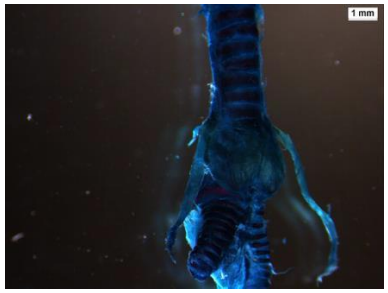
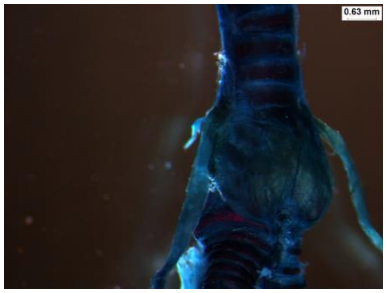
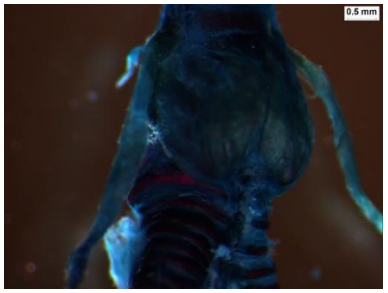
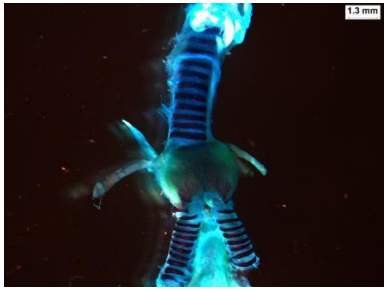
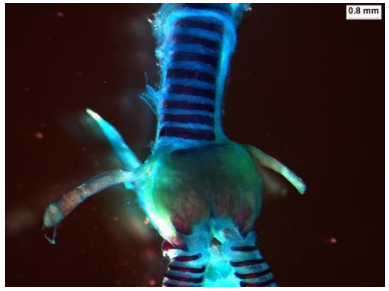
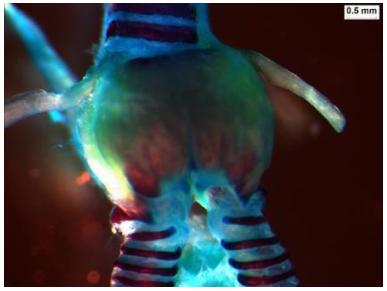
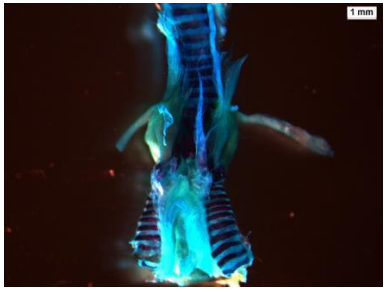
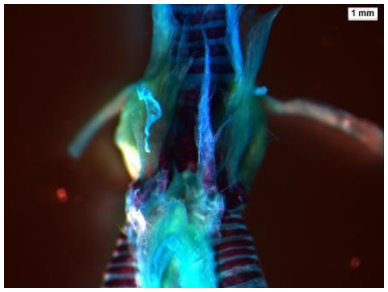
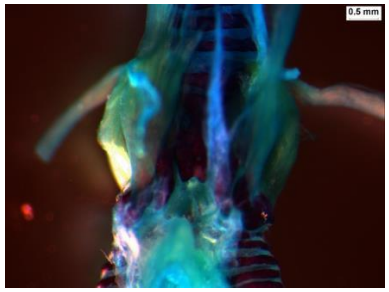
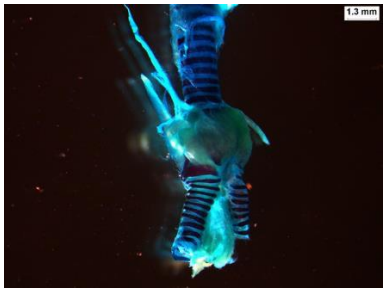
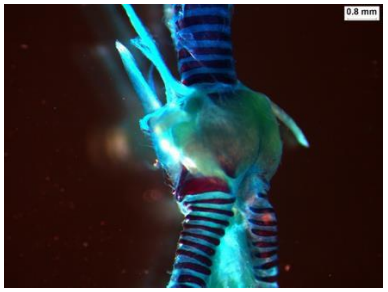
73



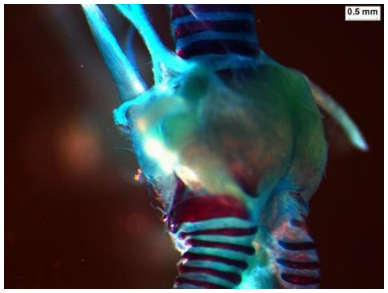
73

73



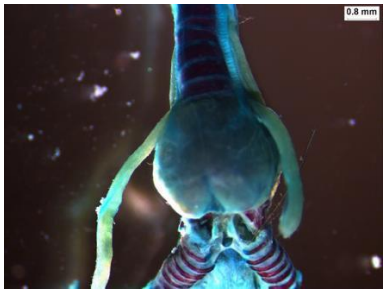
	73	73	73	73
				
Spizocorys sclateri	74b	74	74	74
				
	74	74	74	74
				

74

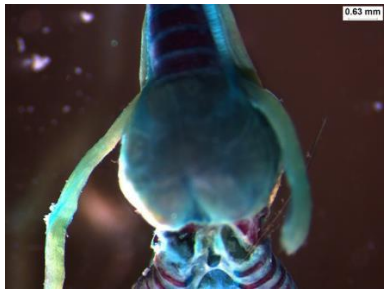


75c

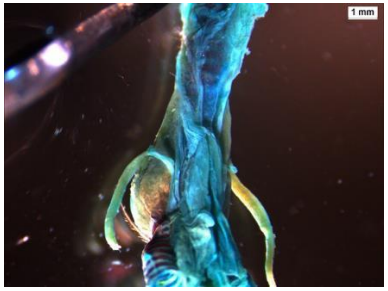
75



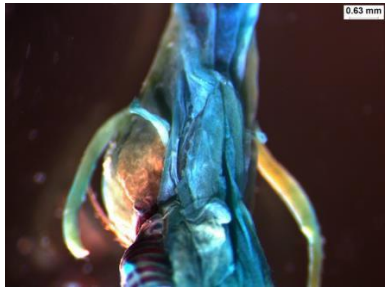
75



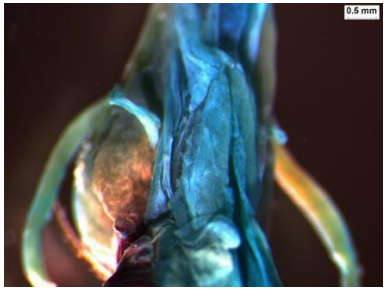
75



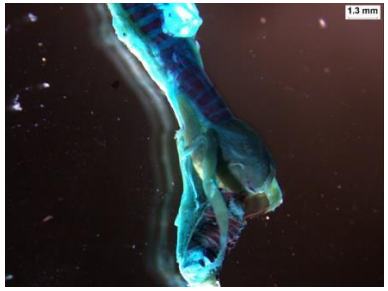
75



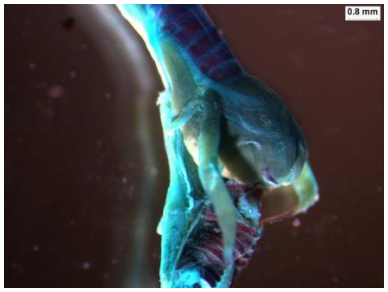
75



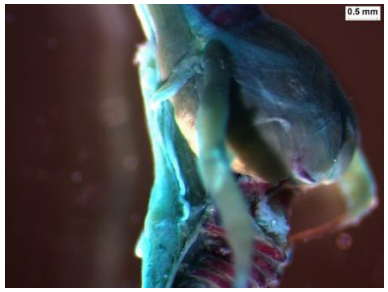
75



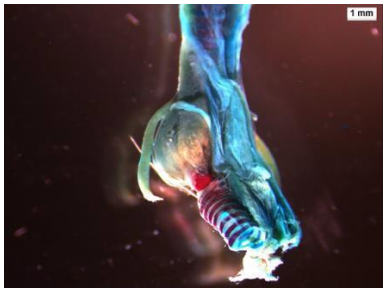
75



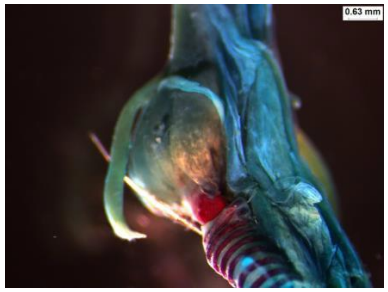
75



75

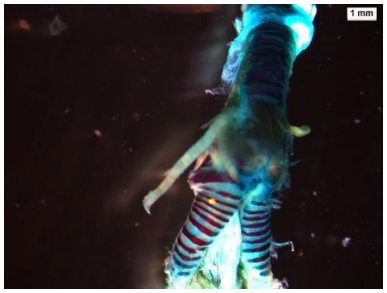


75

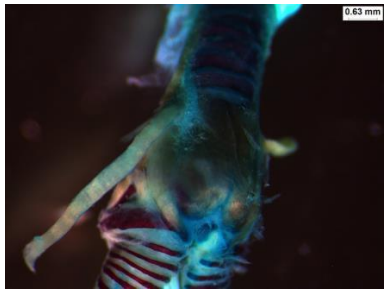


Spizocorys starki (Appendix 2.A.5 a)

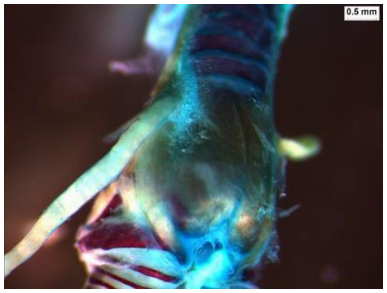
91a



91



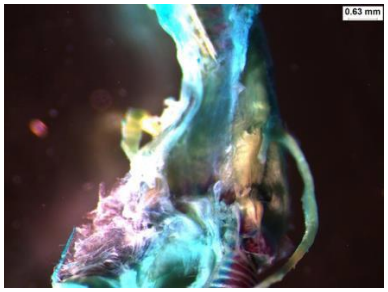
91



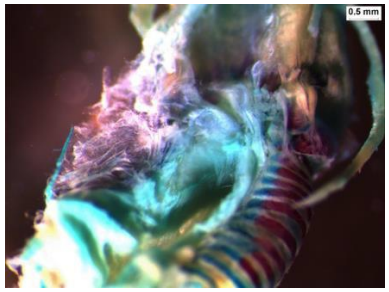
91

Spizocorys starki

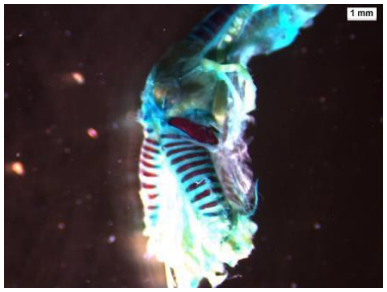
91



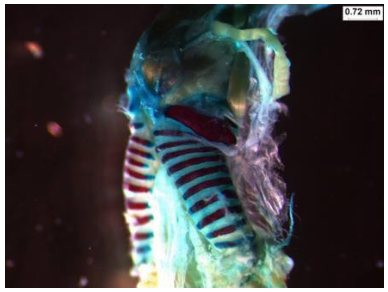
91



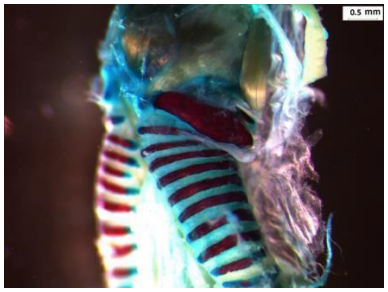
91



91



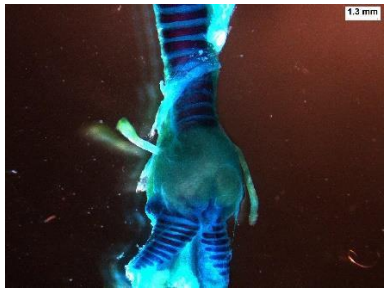
91



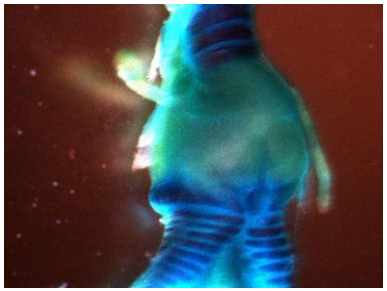
APPENDIX 2.2. GROSS MORPHOLOGY PLATES FOR CLADE B.

Mirafra cheniana (Appendix 2.B.1 a)

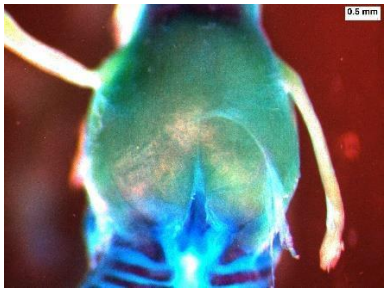
72a



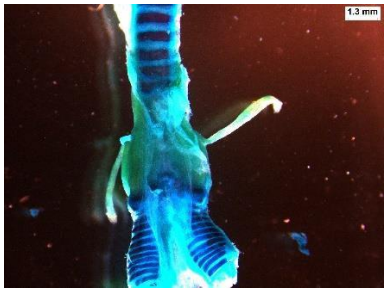
72



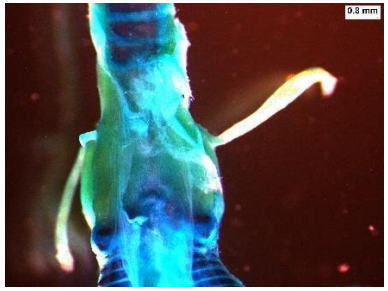
72



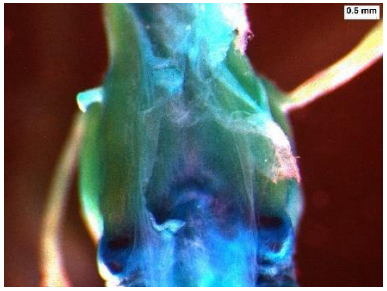
72



72

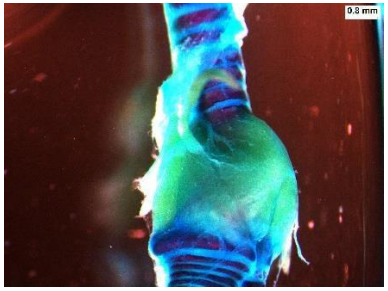


72

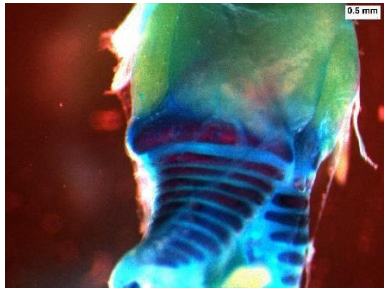


72

72

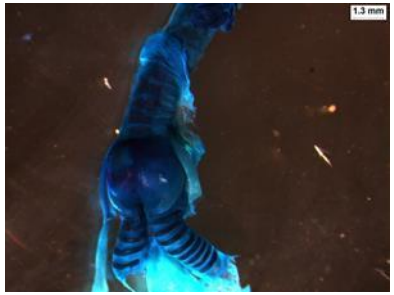


72

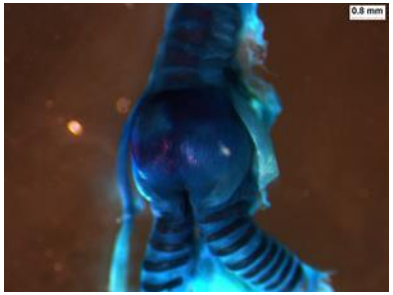


Mirafra africana (Appendix 2.B.2 a-c)

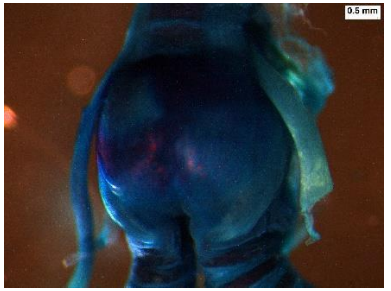
45a



45

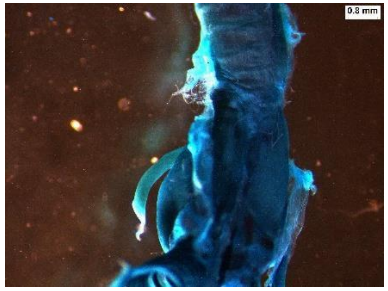


45

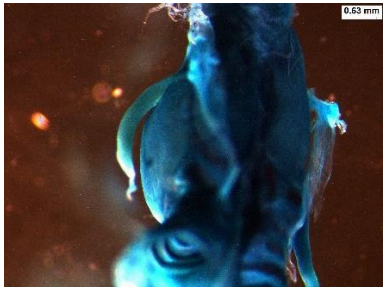


45

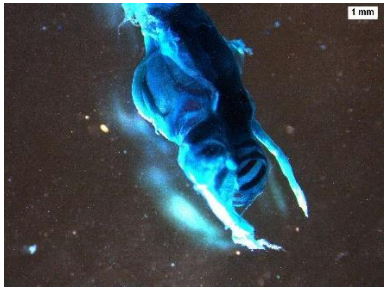
45



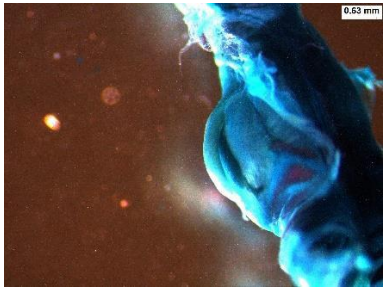
45



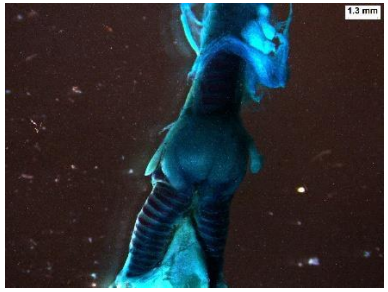
45



45

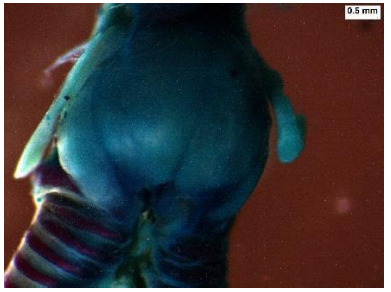


52b

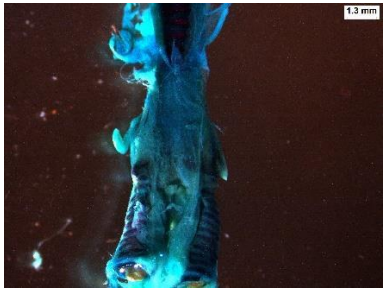


52

52

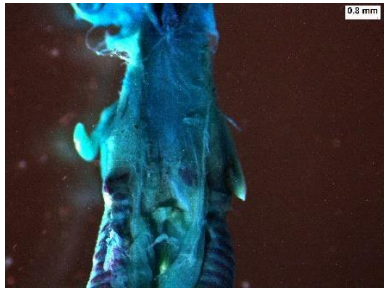


52

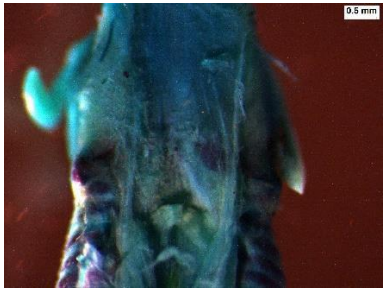


Mirafra africana

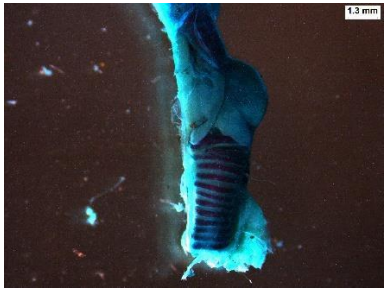
52



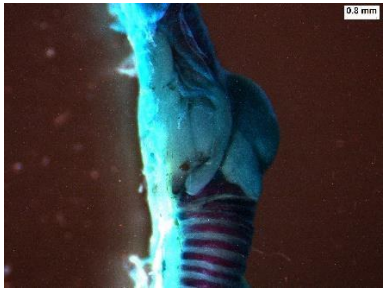
52



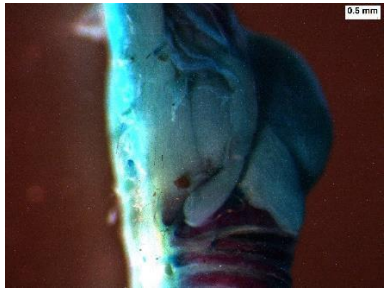
52



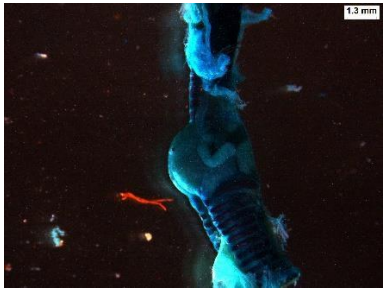
52



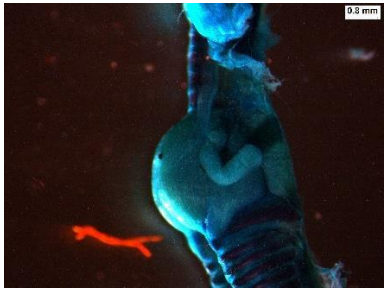
52



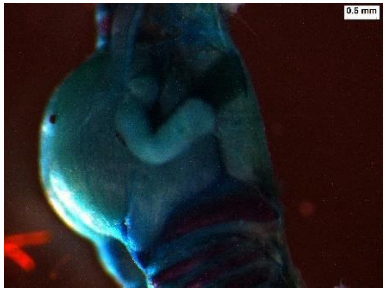
52



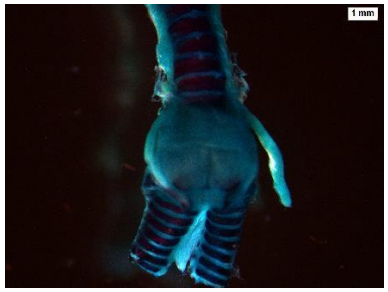
52



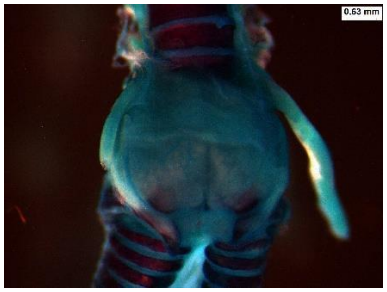
52



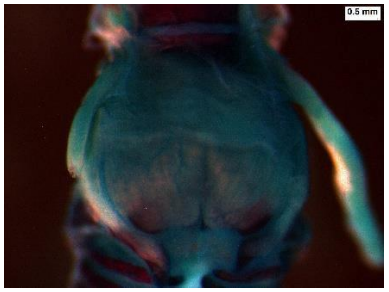
58c



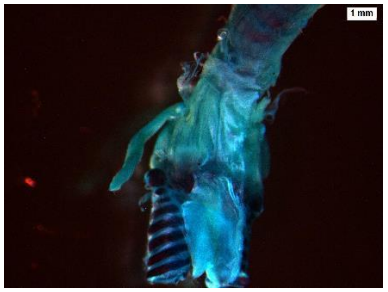
58



58

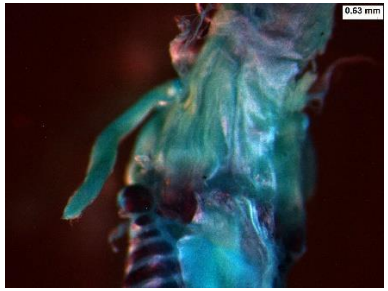


58

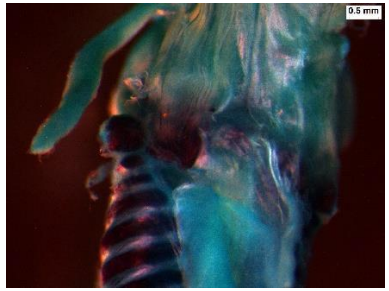


Mirafra africana

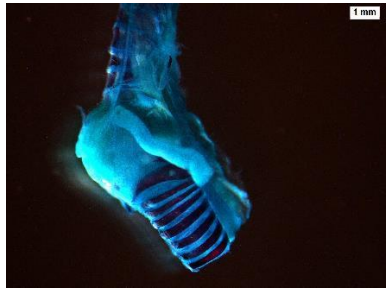
58



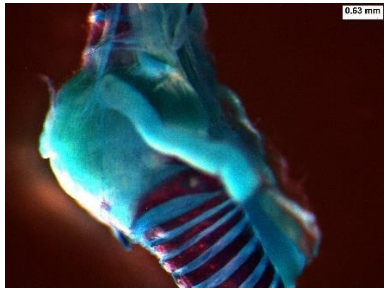
58



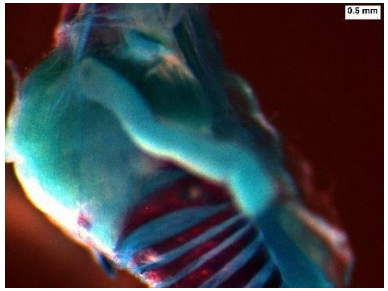
58



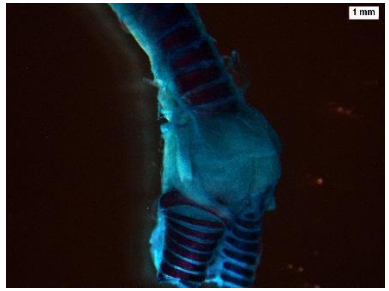
58



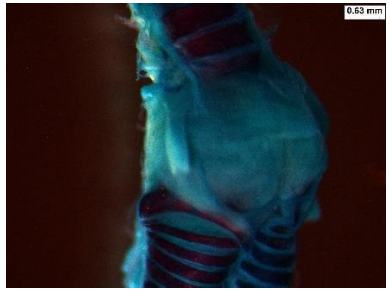
58



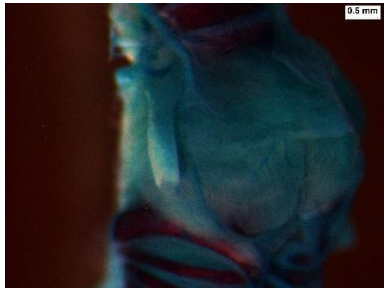
58



58

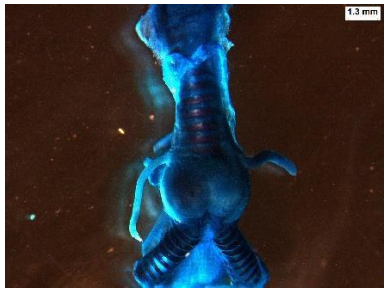


58

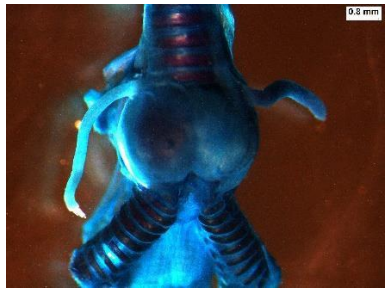


Mirafra fasciolata (Appendix 2.B.3 a-d)

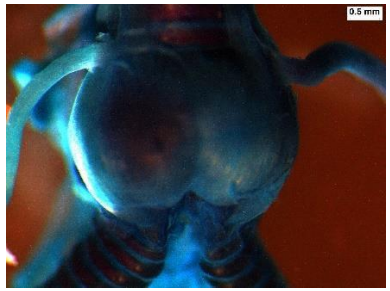
44a



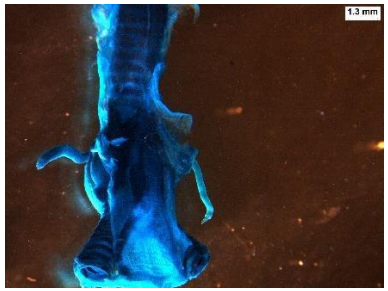
44



44

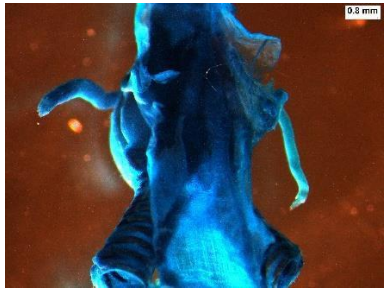


44



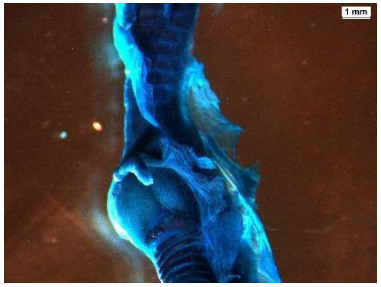
Mirafra fasciolata

44



44

44



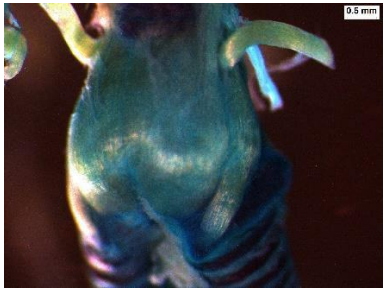
44

44

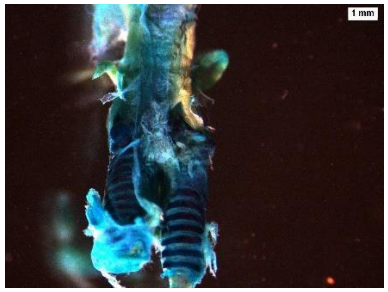
50b

50

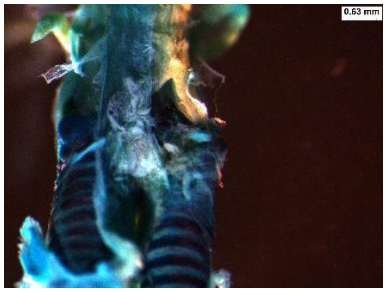
50



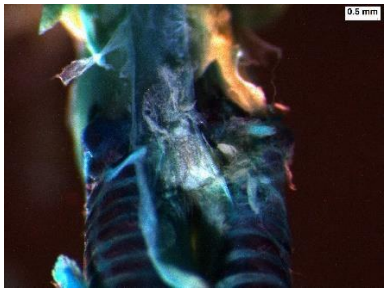
50



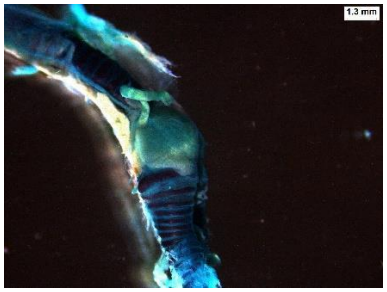
50



50

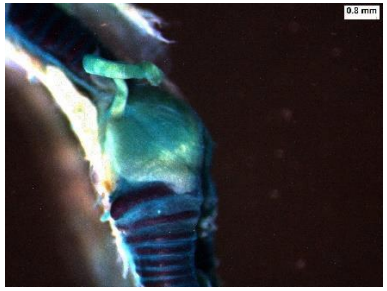


50

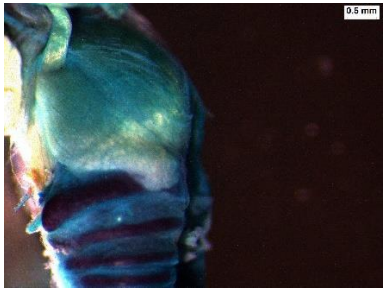


Mirafra fasciolata

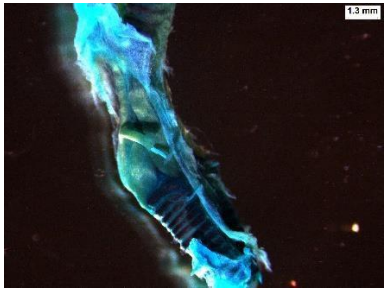
50



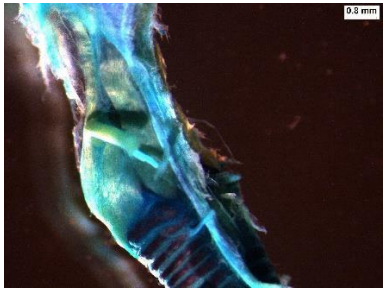
50



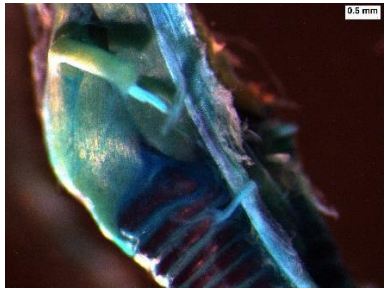
50



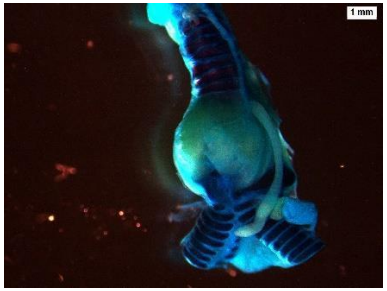
50



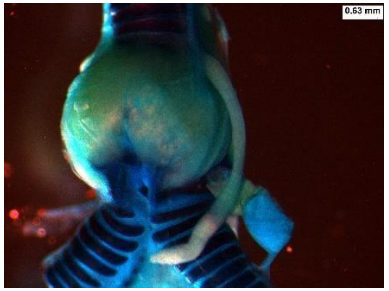
50



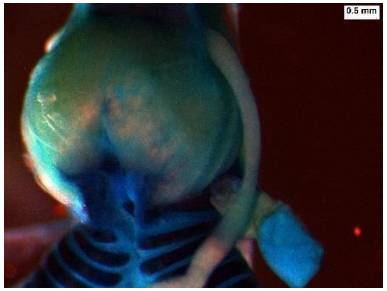
51c



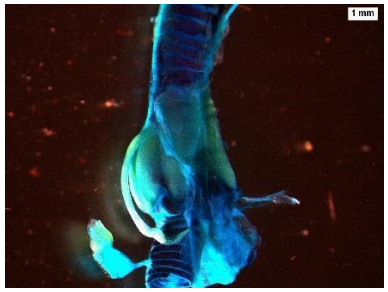
51



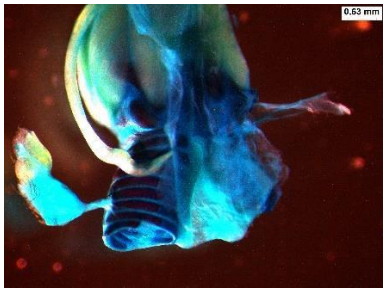
51



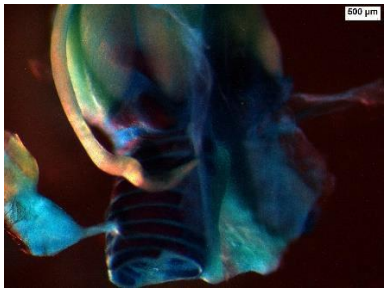
51



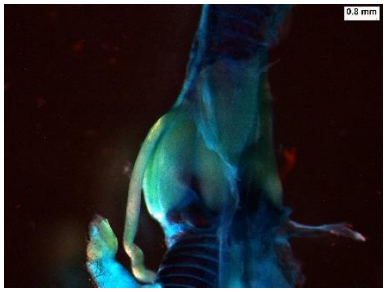
51



51

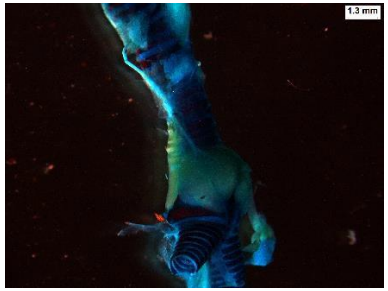


51

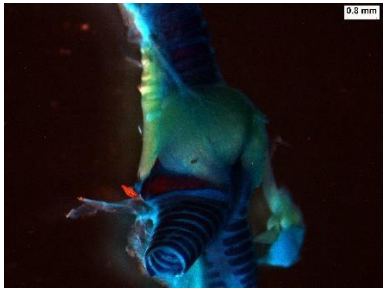


Mirafra fasciolata

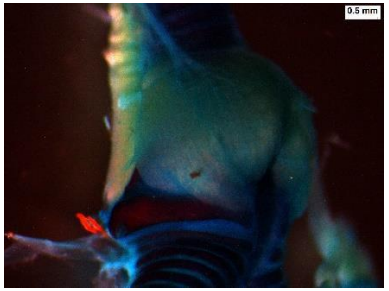
51



51

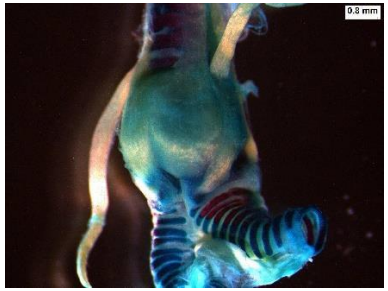


51



56d

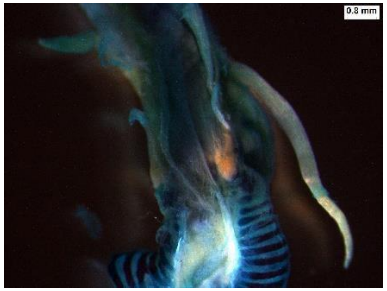
56



56

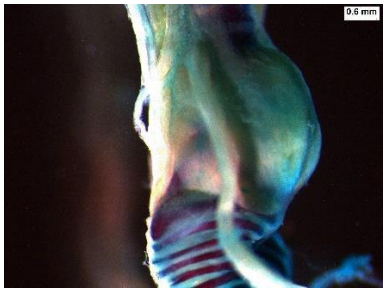
56

56

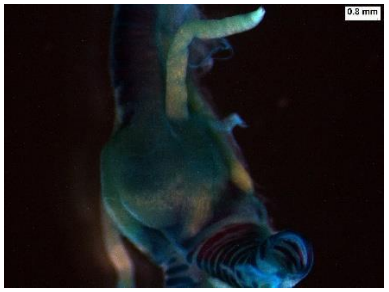


56

56



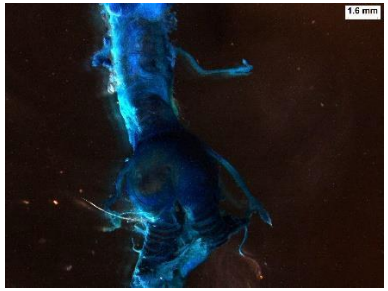
56



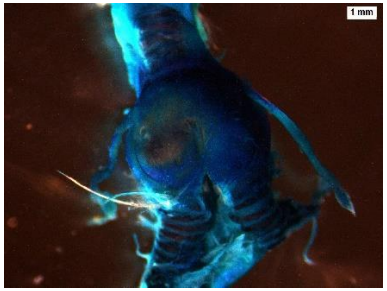
56

Mirafra passerina (Appendix 2.B.4 a-c)

136a

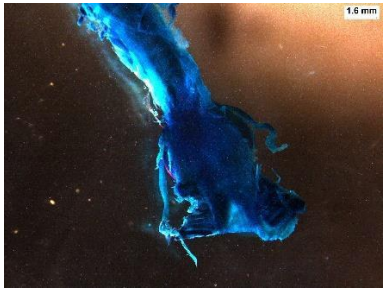


136

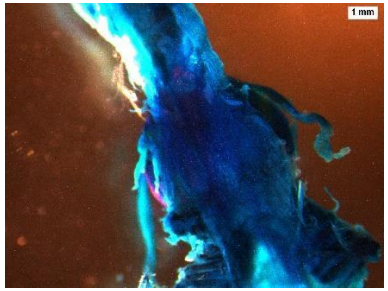


136

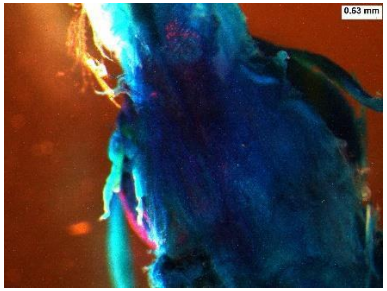
136



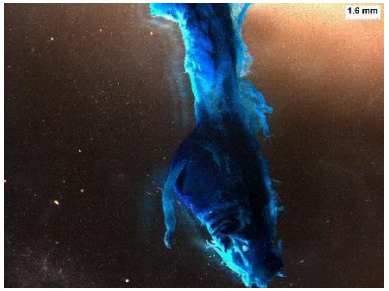
136



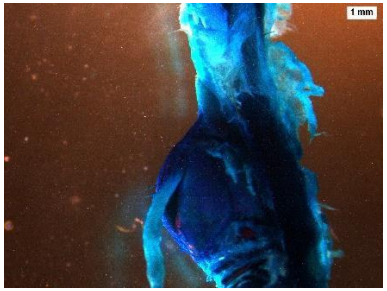
136



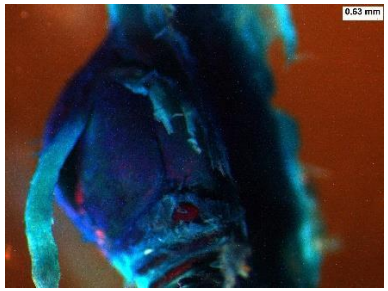
136



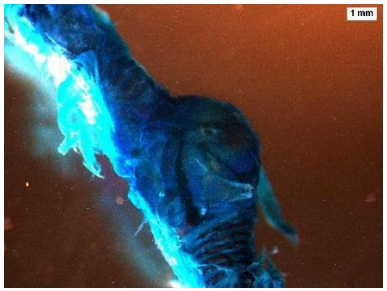
136



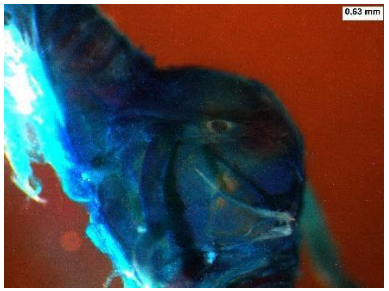
136



136

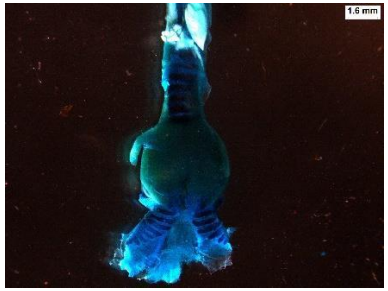


136

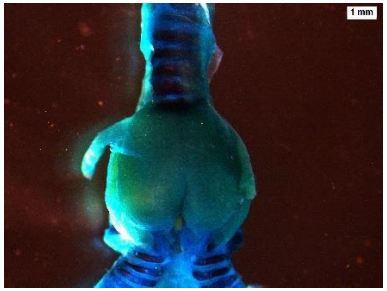


Mirafra passerina

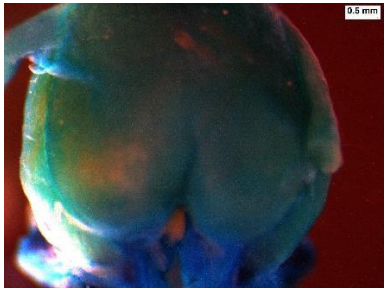
139b



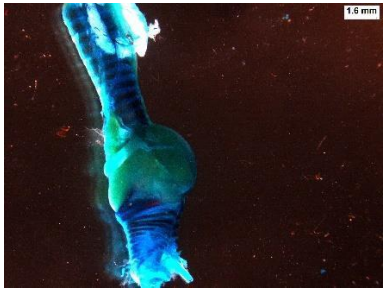
139



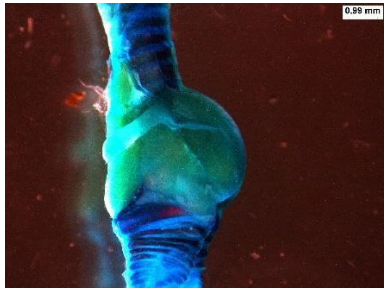
139



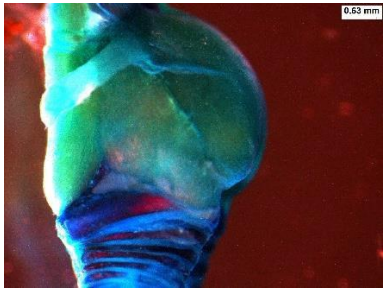
139



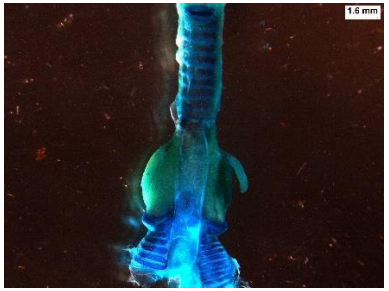
139



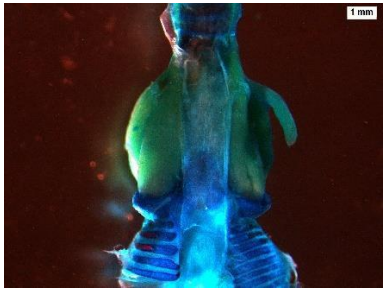
139



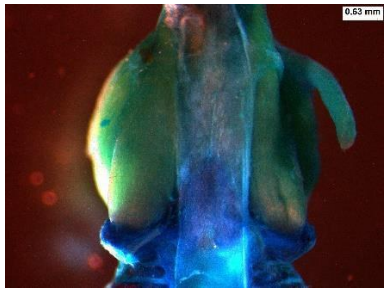
139



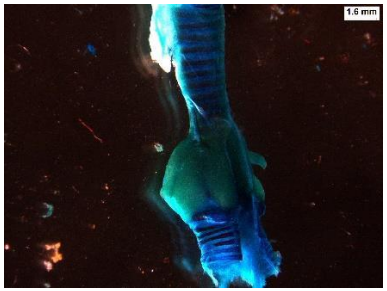
139



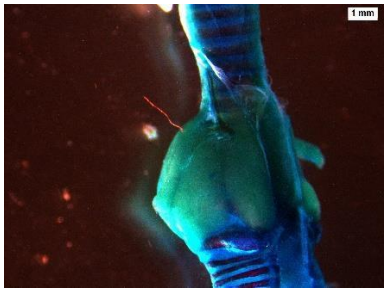
139



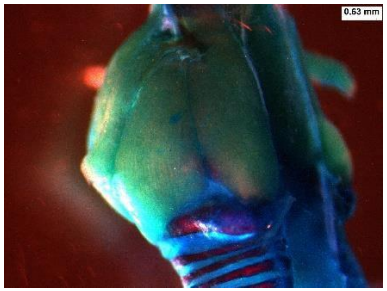
139



139



139

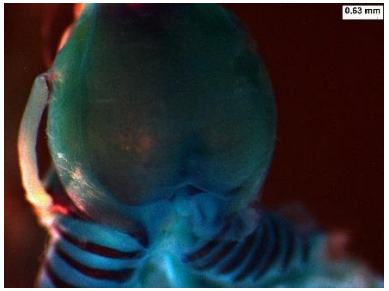


Mirafra passerina

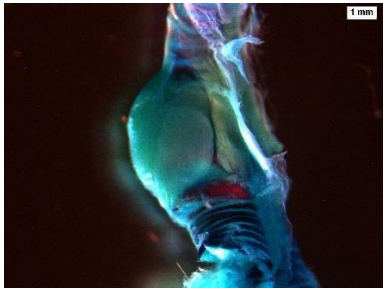
140c

140

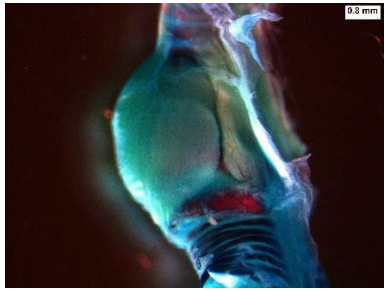
140



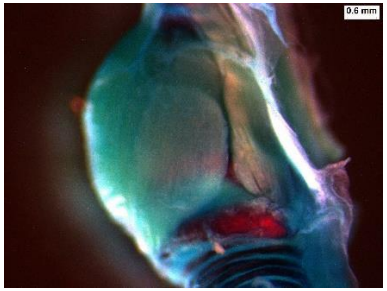
140



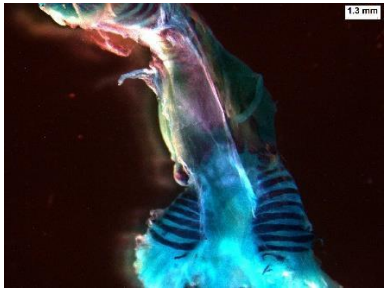
140



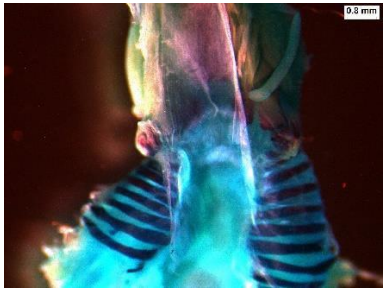
140



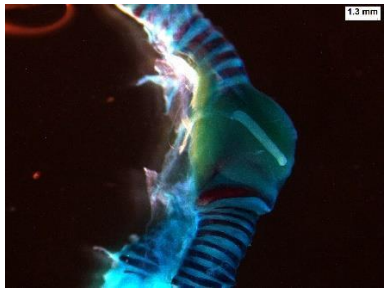
140



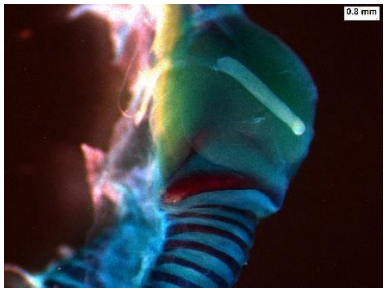
140



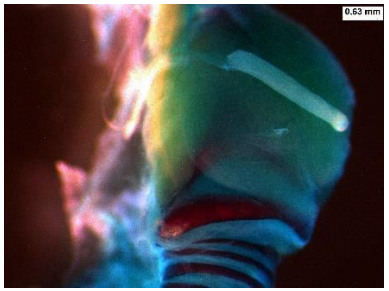
140



140



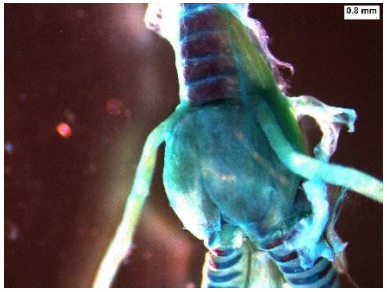
140



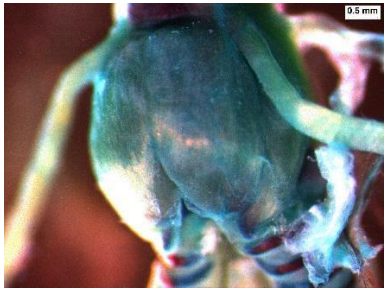
Calendulauda africanoides (Appendix 2.B.5 a-b)

76a

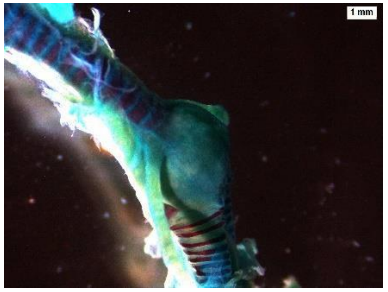
76



76

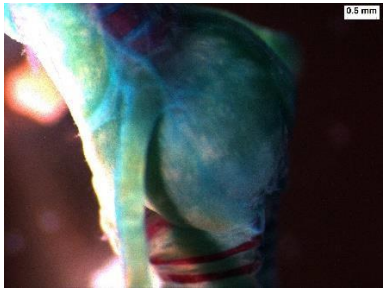


76

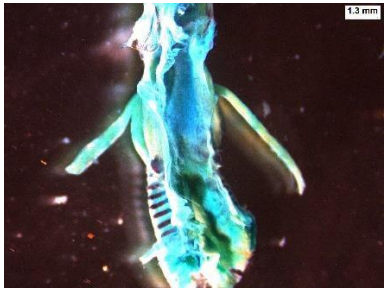


76

76

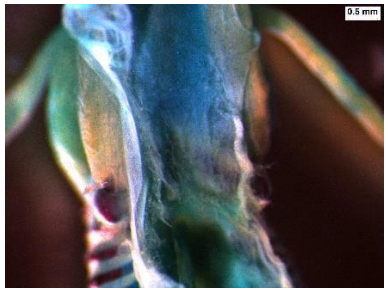


76

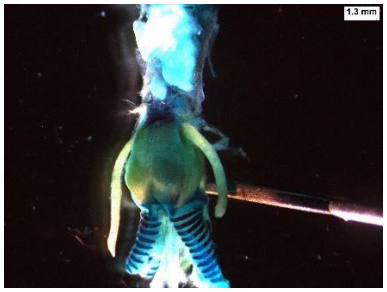


76

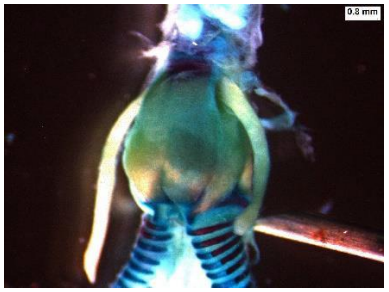
76



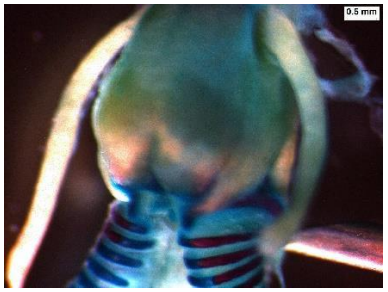
99b



99

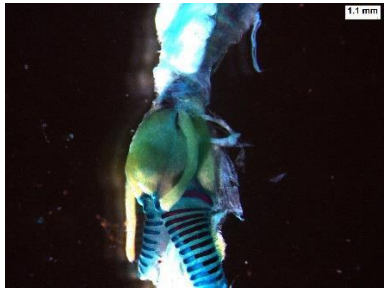


99

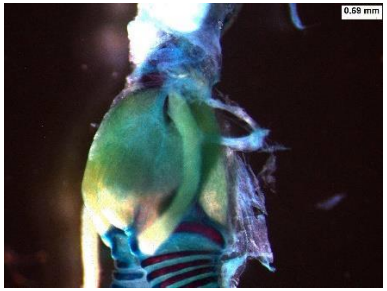


Calendulauda africanoides

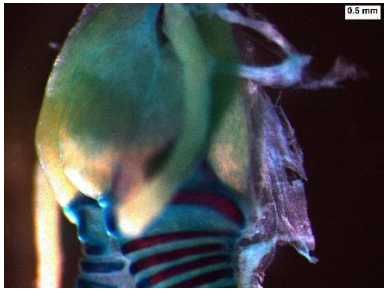
99



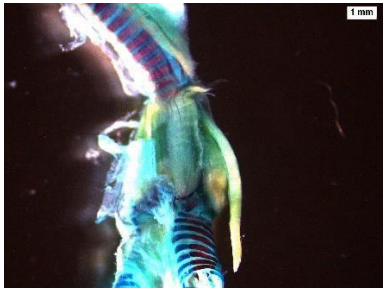
99



99

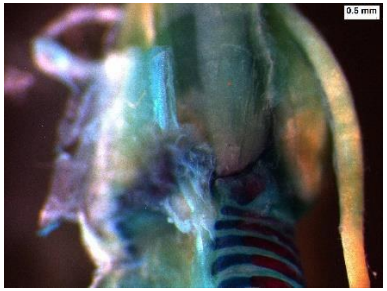


99



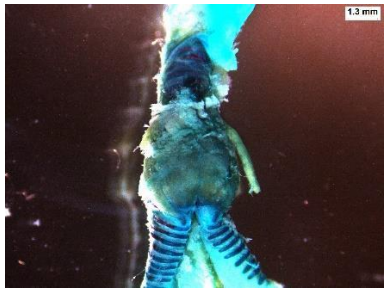
99

99

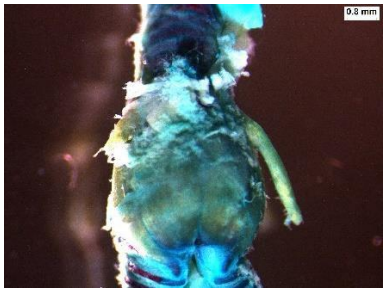


Calendulauda albescens (Appendix 2.B.6 a-b)

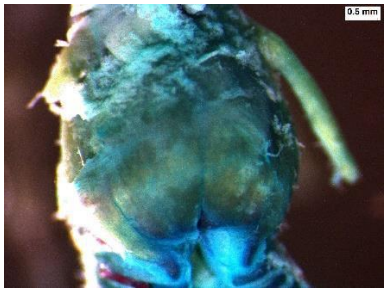
86a



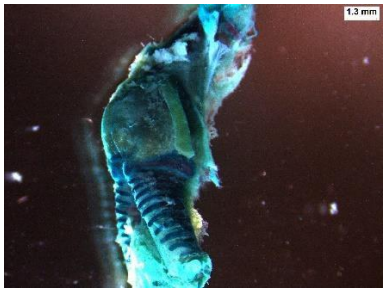
86

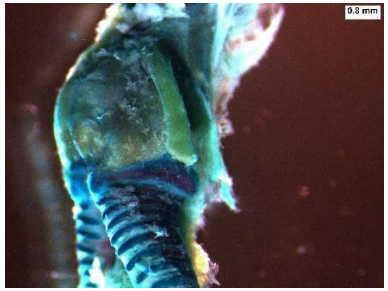
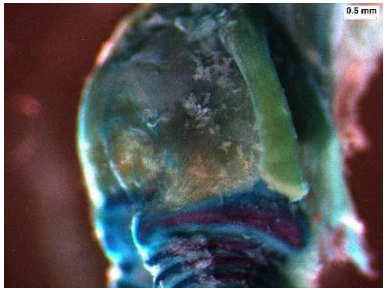
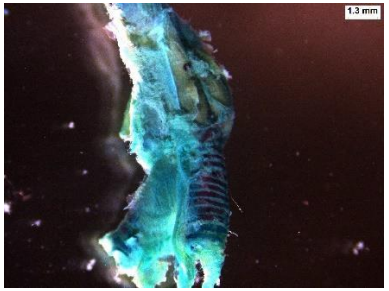
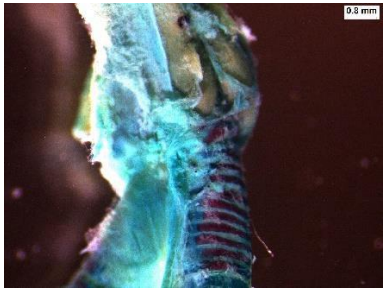
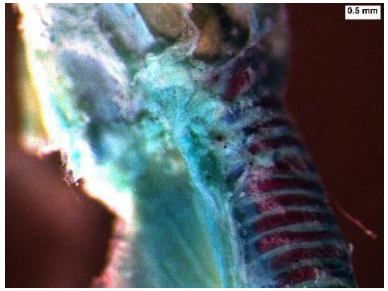
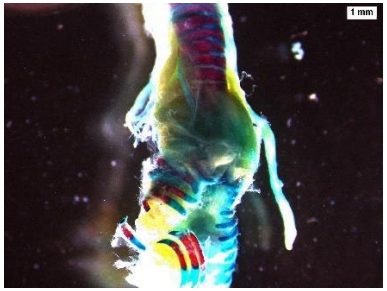
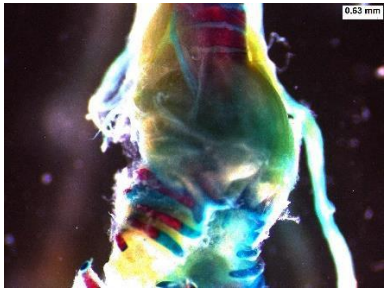
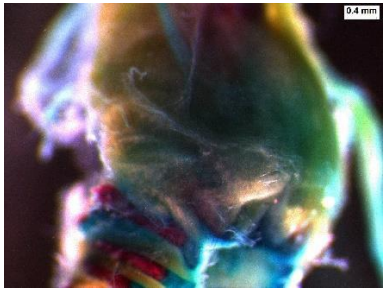
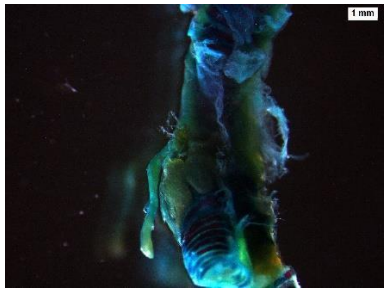
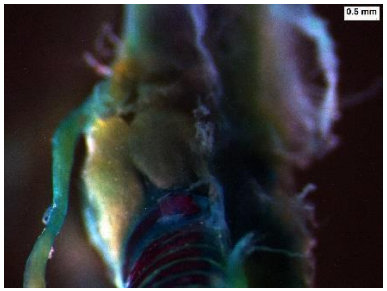


86



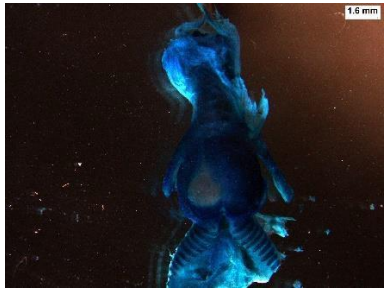
86



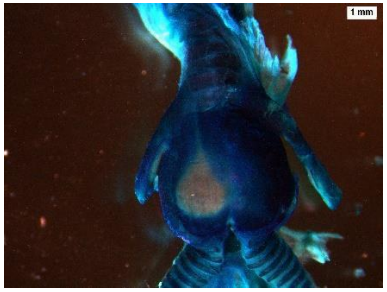
	86	86	86	86
				
Calendulauda albescens	86	100b	100	100
				
	100	100		
				

Calendulauda burra (Appendix 2.B.7 a-c)

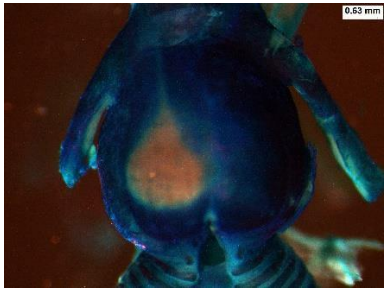
87a



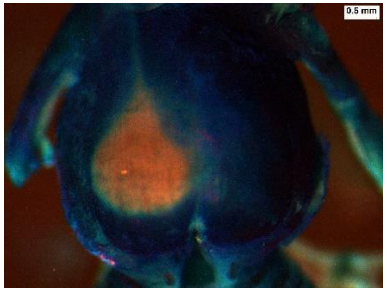
87



87

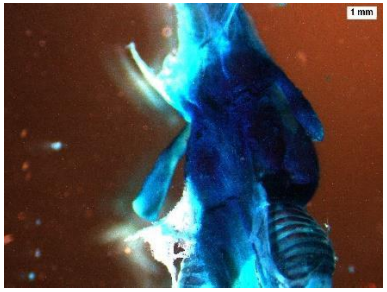


87

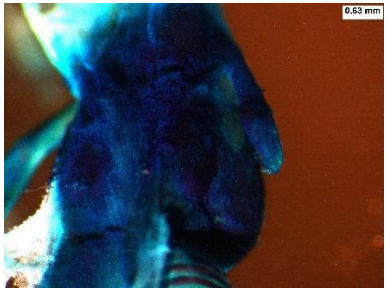


87

87

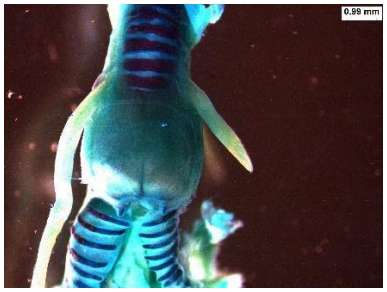


87

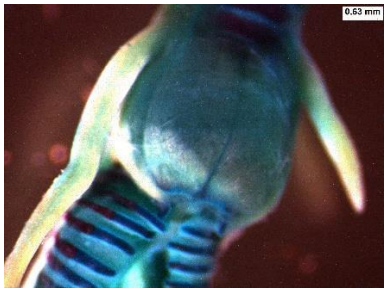


88b

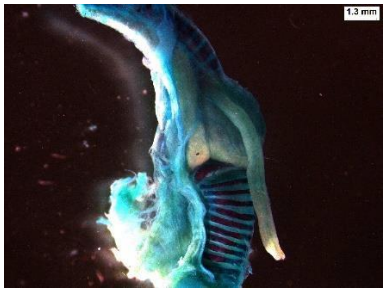
88



88

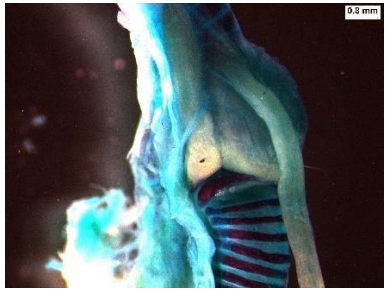


88



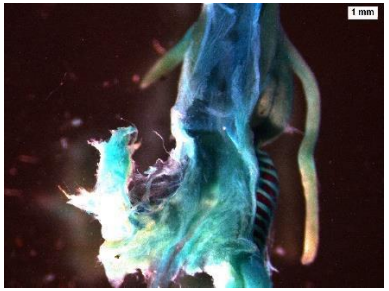
Calendulauda burra

88

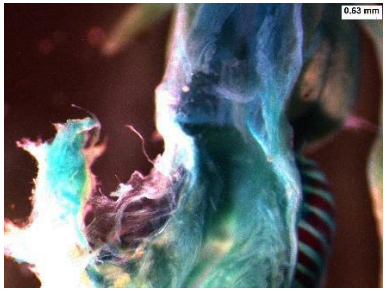


88

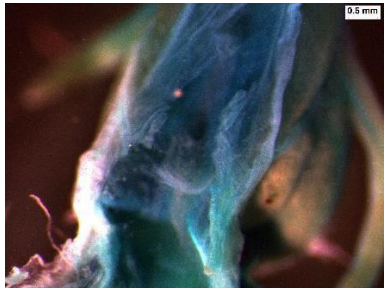
88



88

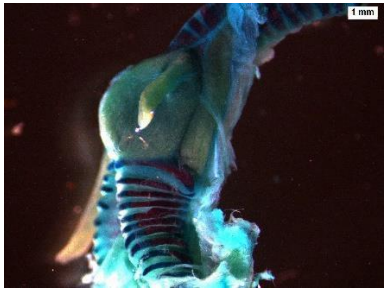


88

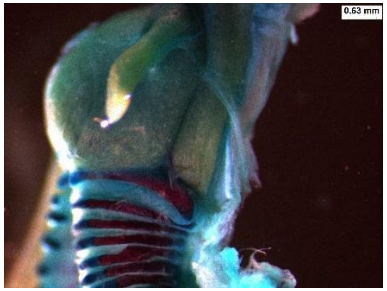


88

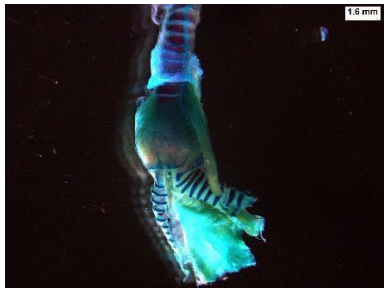
88



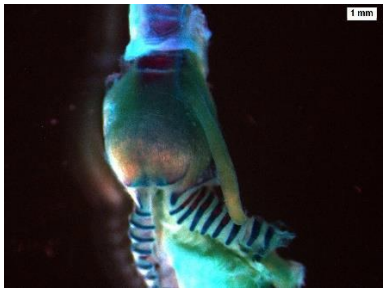
88



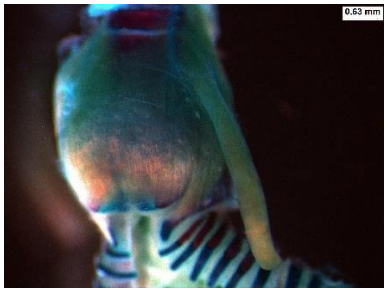
89c



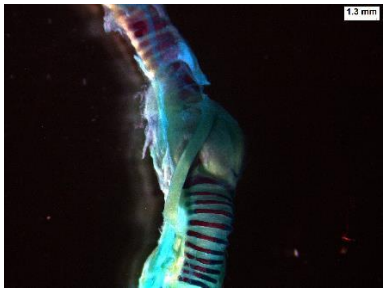
89



89

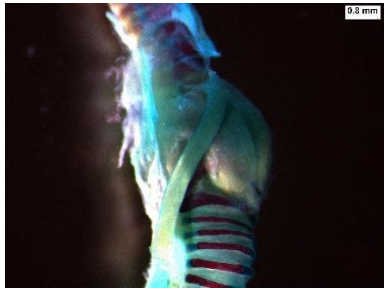


89

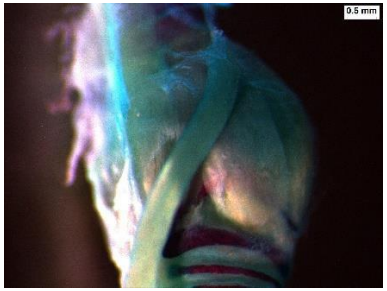


Calendulauda burra

89

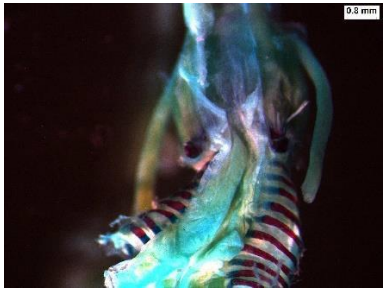


89

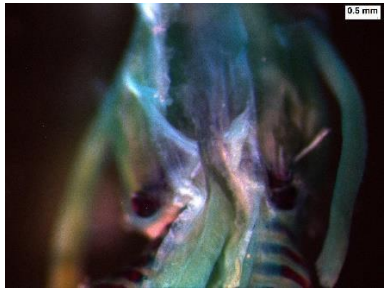


89

89

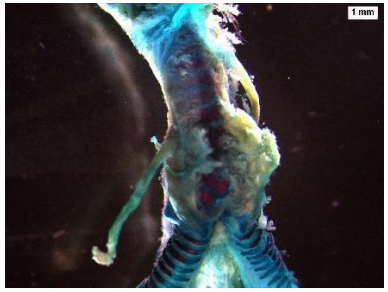


89



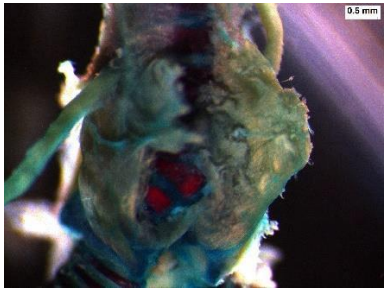
Calendulauda erythrochlamys (Appendix 2.B.8 a)

96a



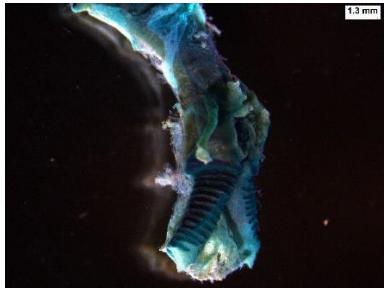
96

96

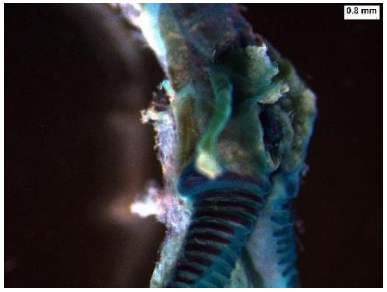


96

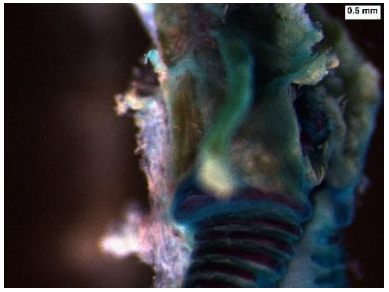
96



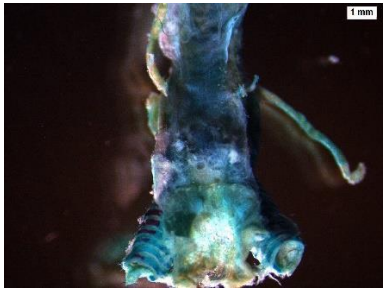
96



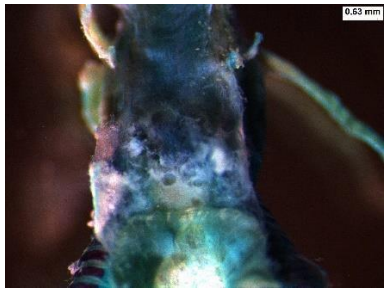
96



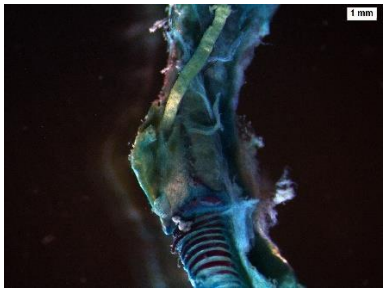
96



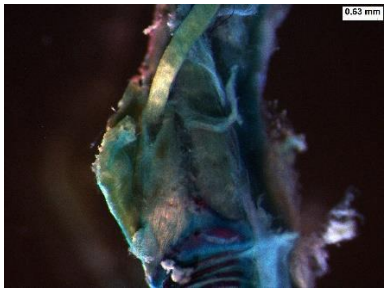
96



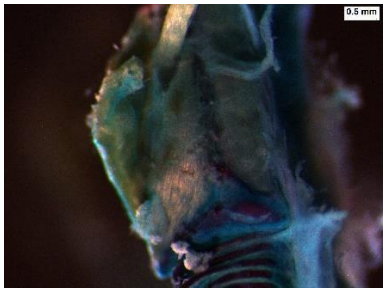
96



96

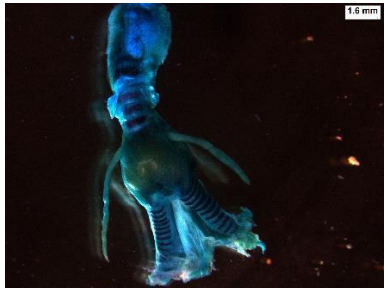


96



Calendulauda sabota (Appendix 2.B.9 a-d)

92a

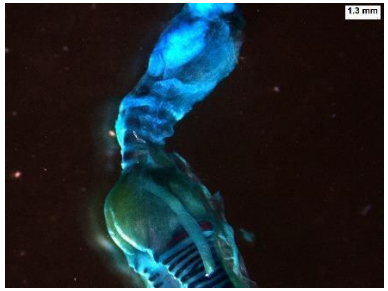


92

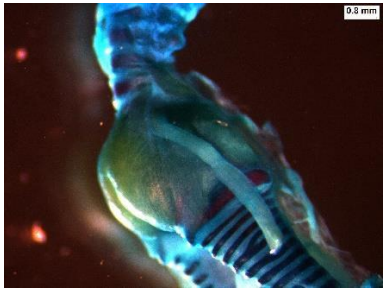
92

92

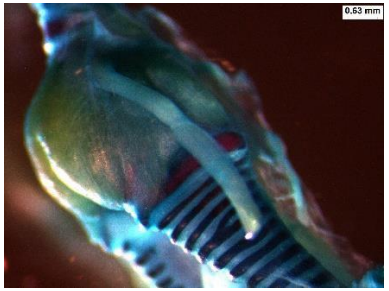
92



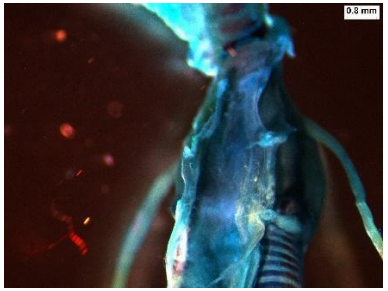
92



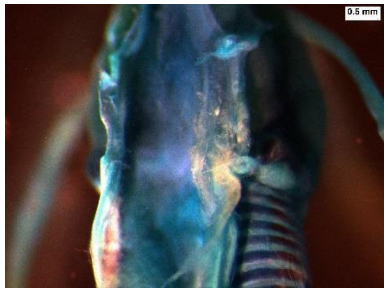
92



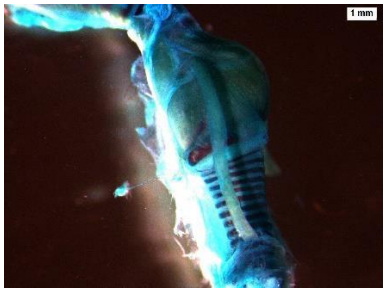
92



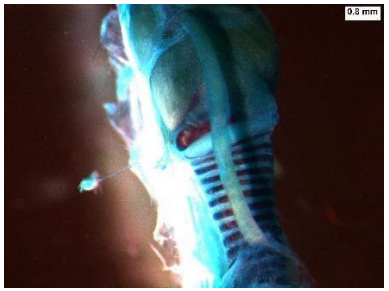
92



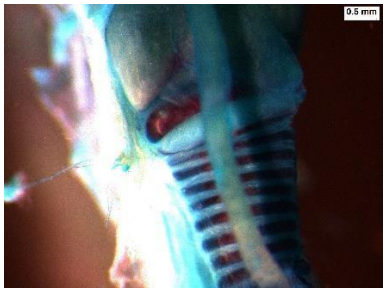
92

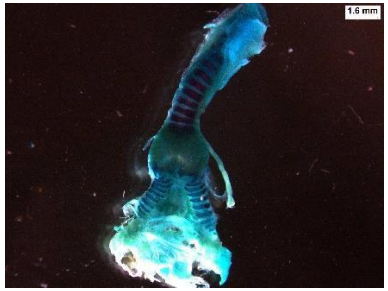
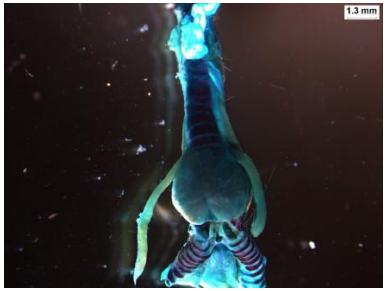
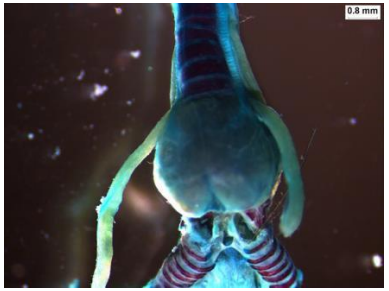
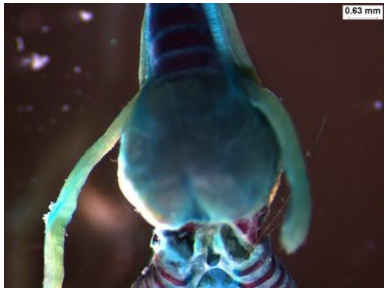
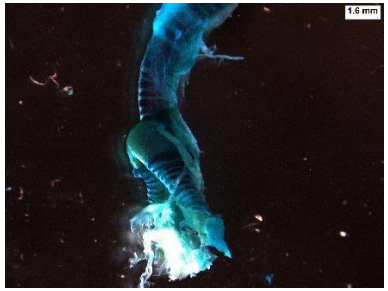
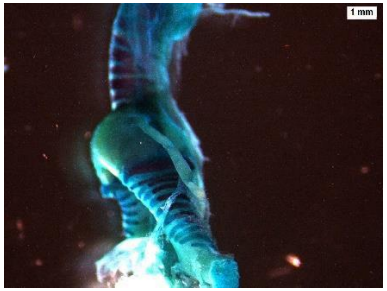
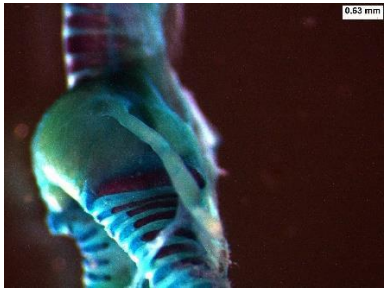
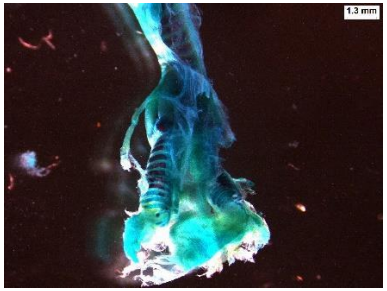
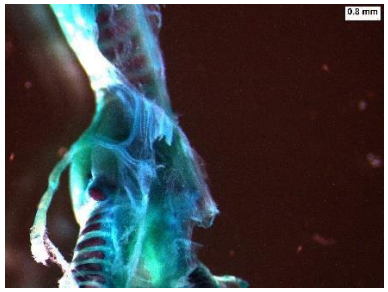
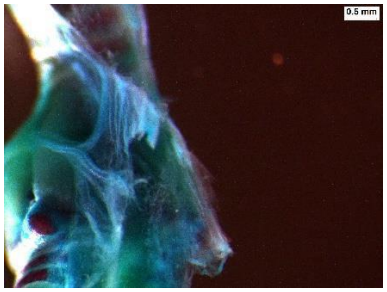
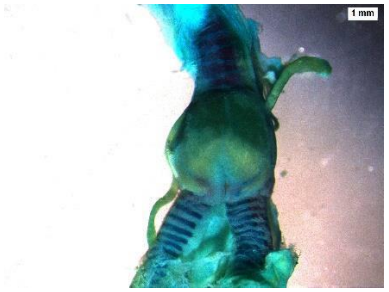
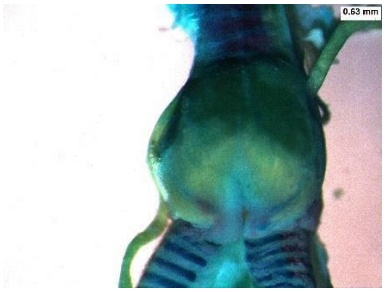


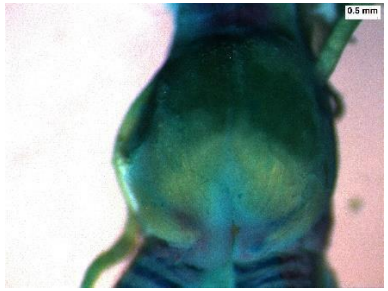
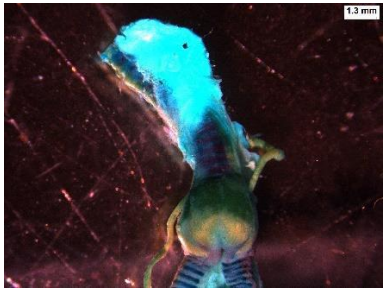
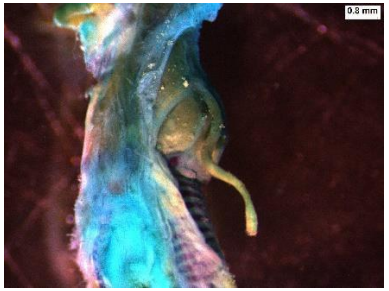
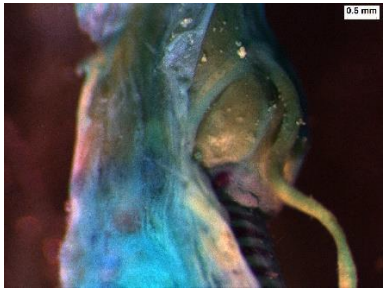
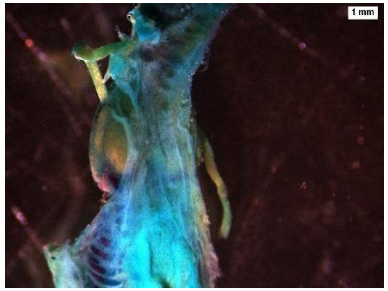
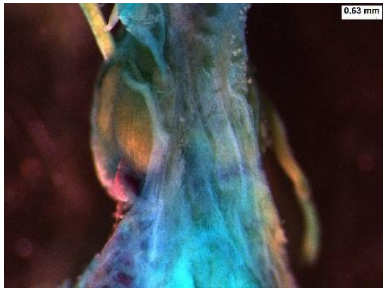
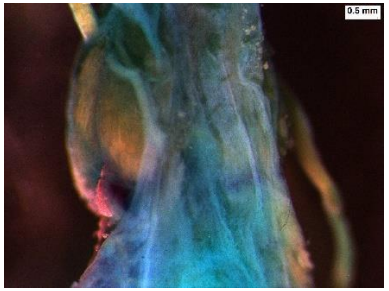
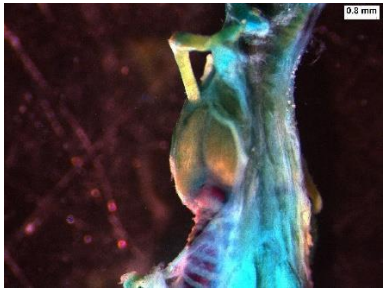
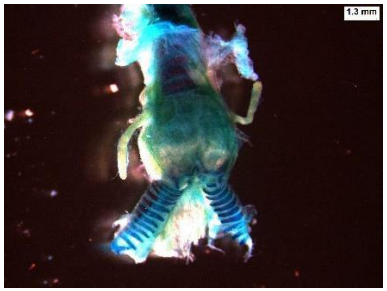
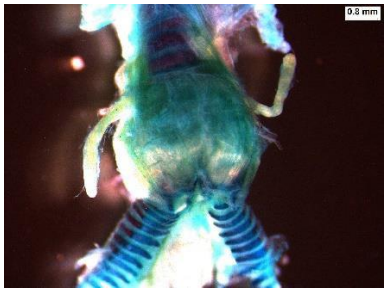
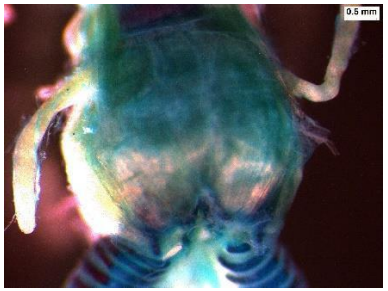
92



92

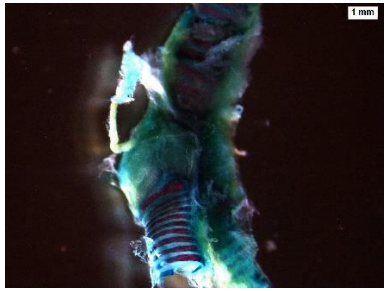


	94b	94	94	94
				
Calendulauda sabota	94	94	94	94
				
	94	94	95c	95
				

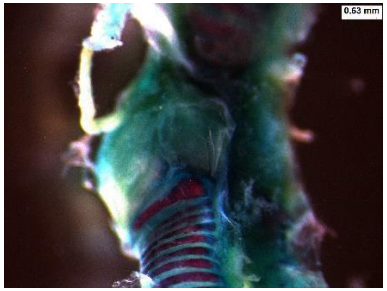
	95	95	95	95
				
Calendulauda sabota	95	95	95	95
				
	95	97d	97	97
				

Calendulauda sabota

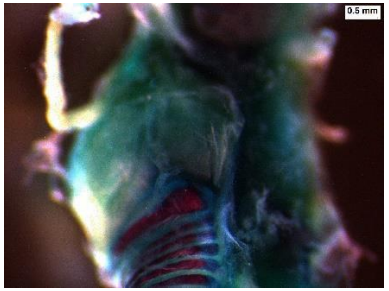
97



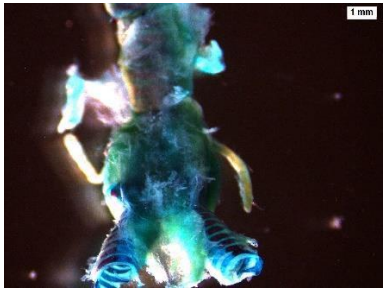
97



97

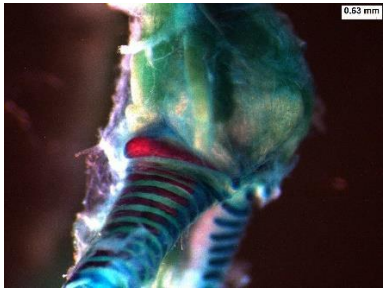


97

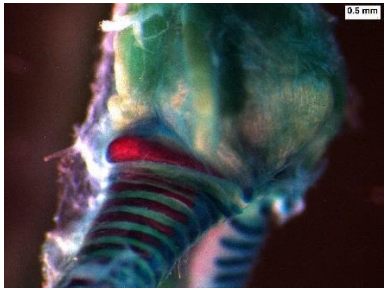


97

97



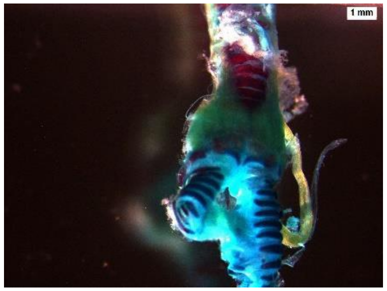
97



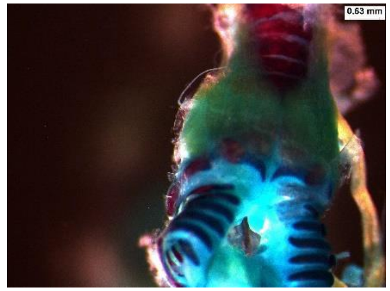
APPENDIX 2.3. GROSS MORPHOLOGY PLATES FOR CLADE C.

Certhilauda subcoronata (Appendix 2.C.1 a-b)

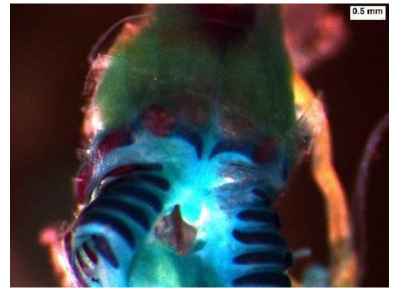
41a



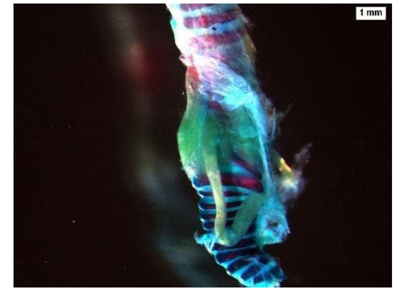
41



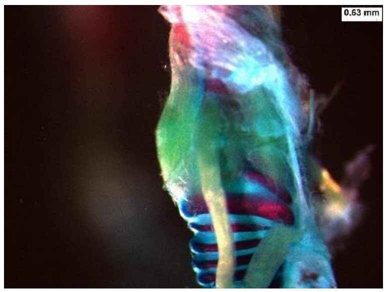
41



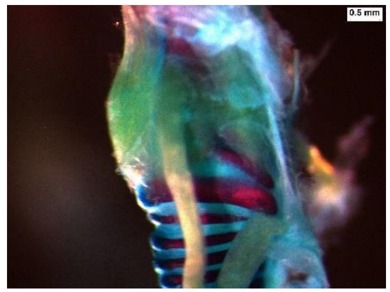
41



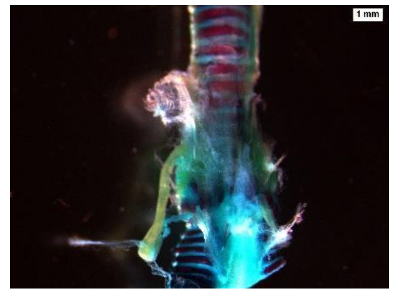
41



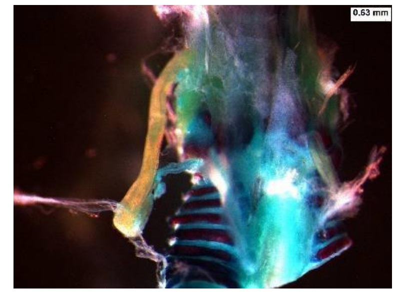
41



41

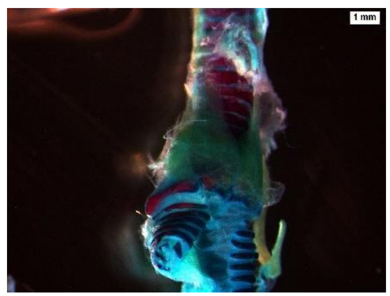


41

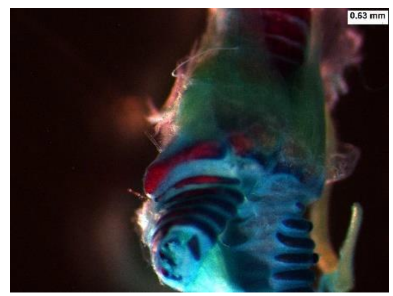


41

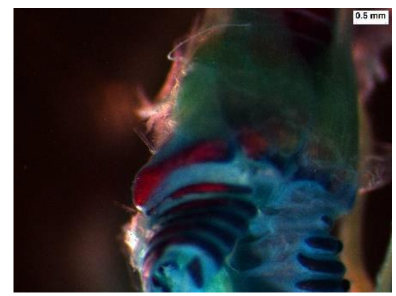
41



41

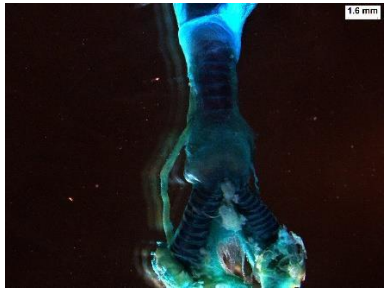


41

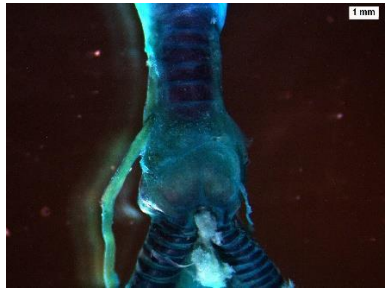


Certhilauda subcoronata

93b

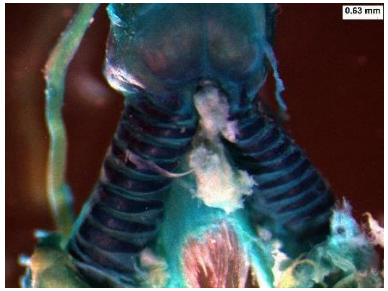


93

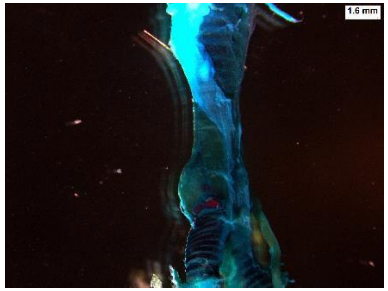


93

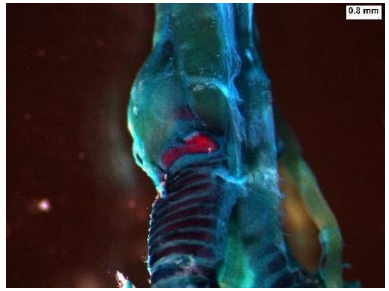
93



93

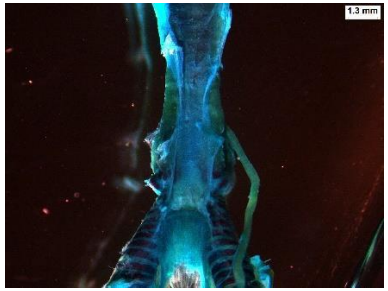


93

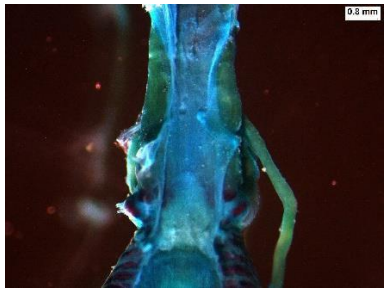


93

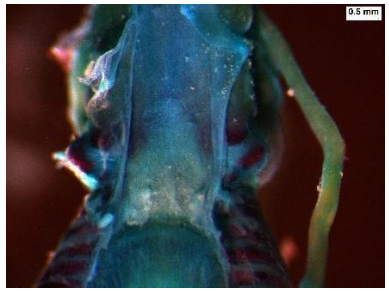
93



93

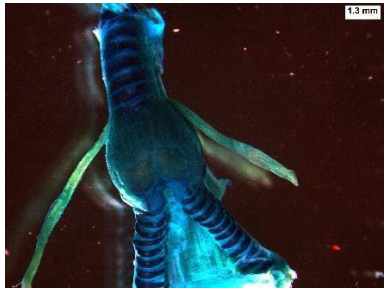


93

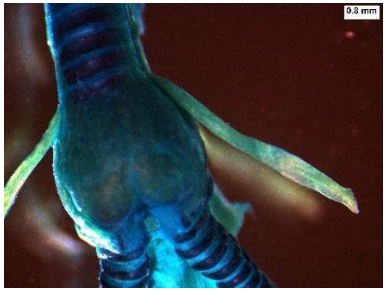


Certhilauda curvirostris (Appendix 2.C.2 a-b)

78a



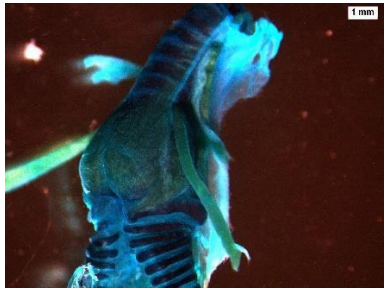
78



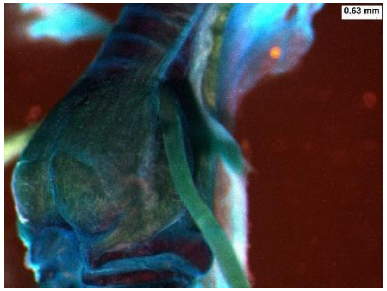
78

78

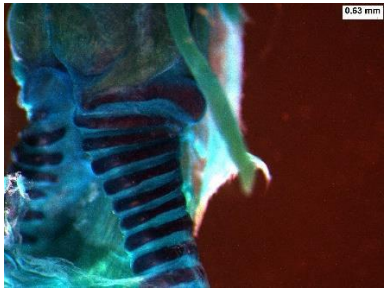
78



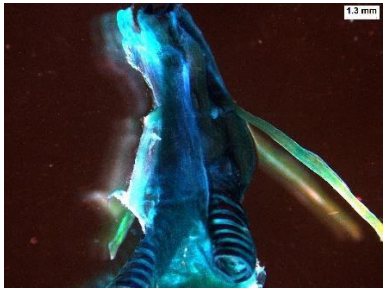
78



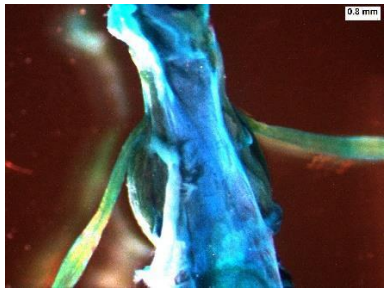
78



78



78

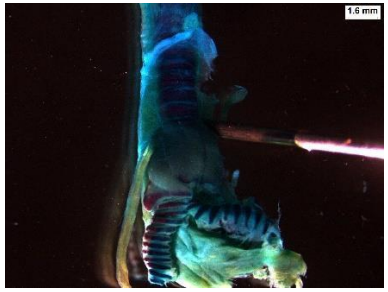


78

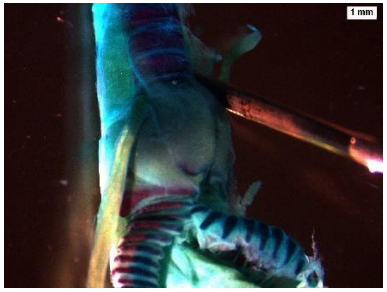
78

Certhilauda curvirostris

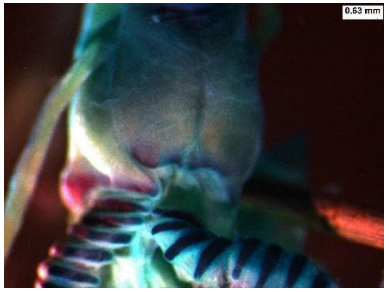
79b



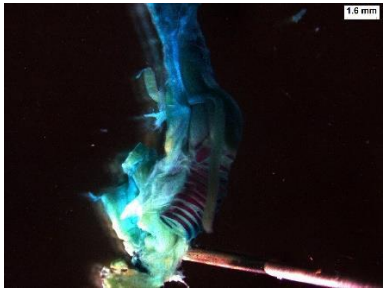
79



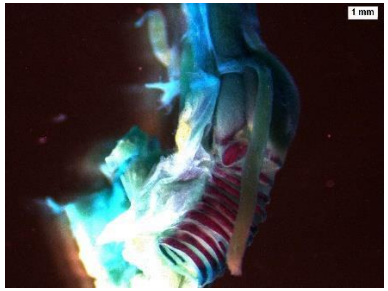
79



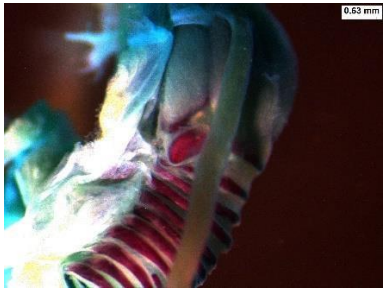
79



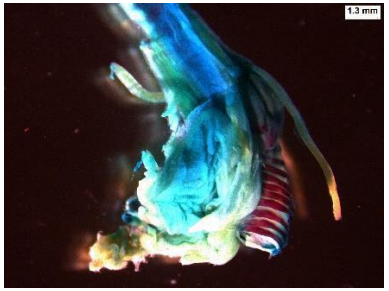
79



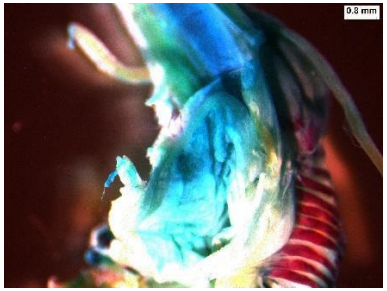
79



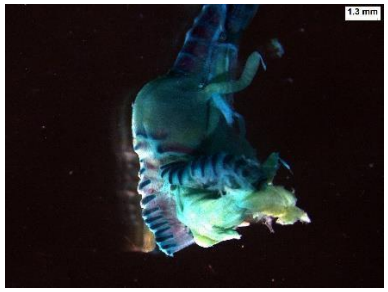
79



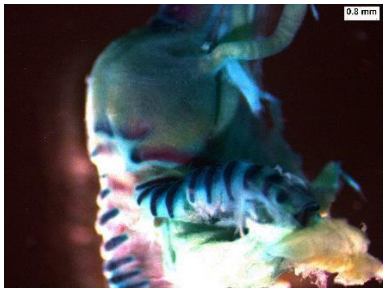
79



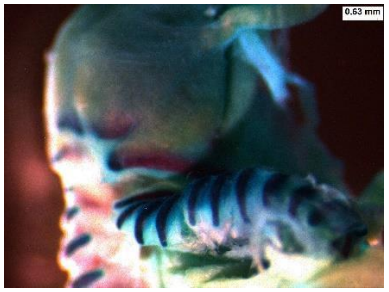
79



79

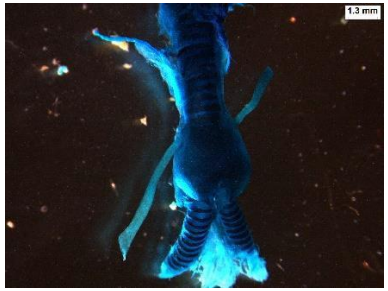


79

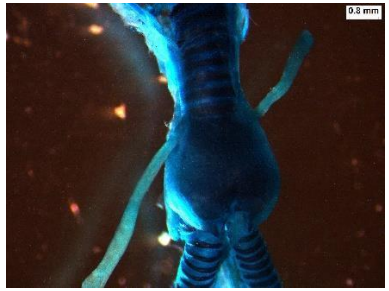


Eremopterix verticalis (Appendix 2.C.3 a-c)

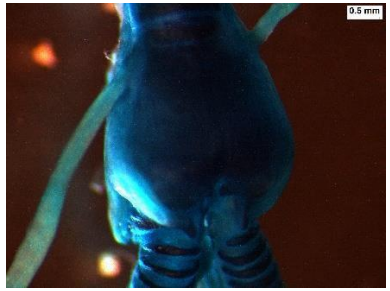
85a



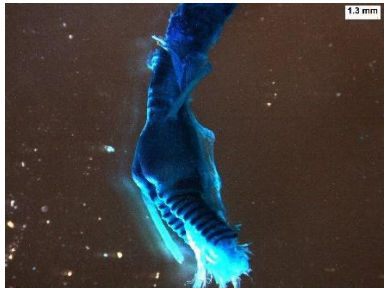
85



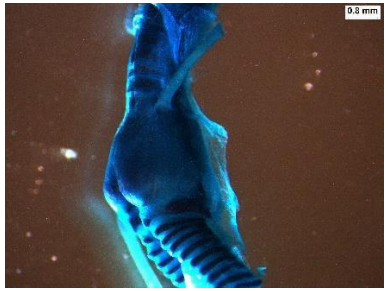
85



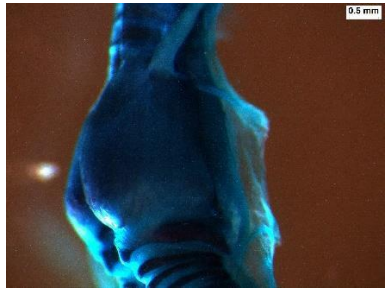
85



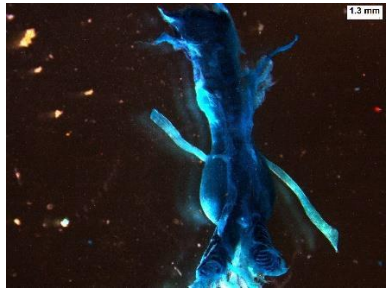
85



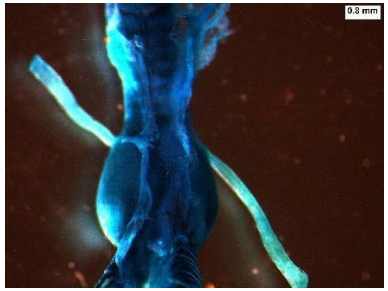
85



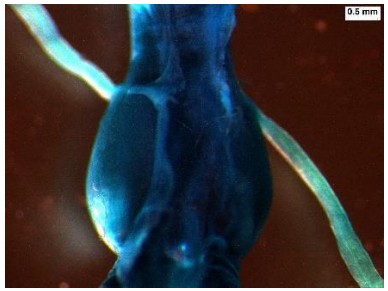
85



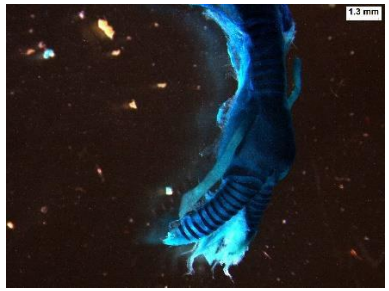
85



85



85

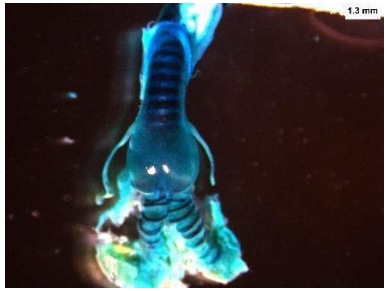


85

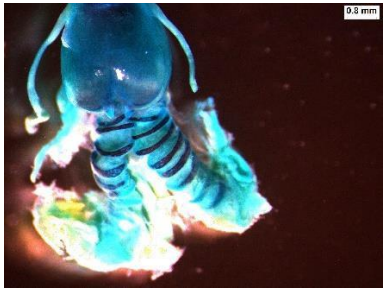
85

Eremopterix verticalis

90b

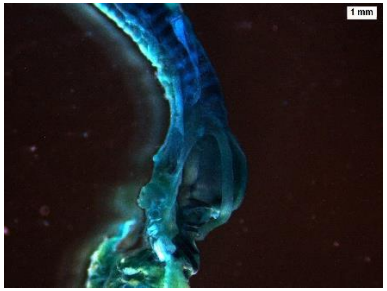


90

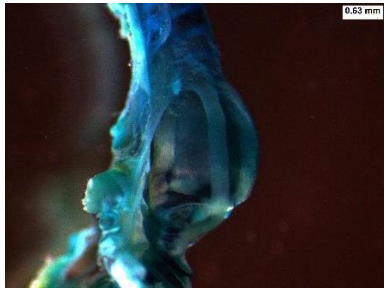


90

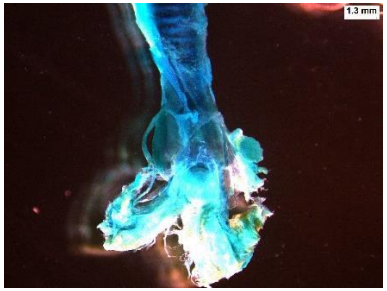
90



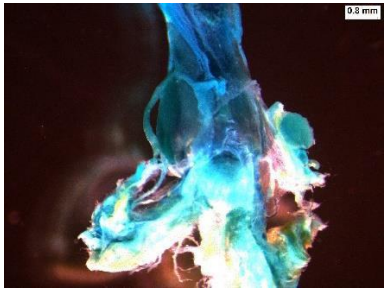
90



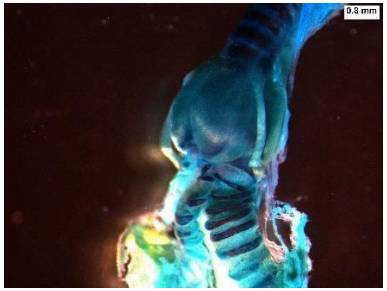
90



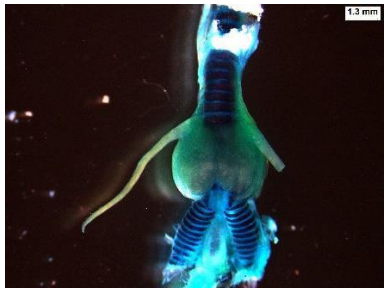
90



90



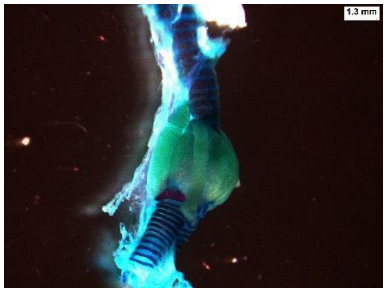
98c

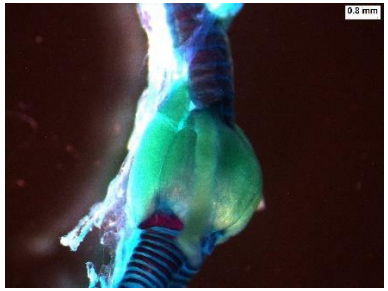
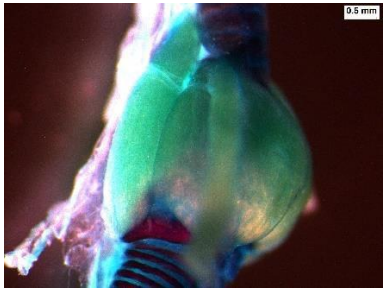
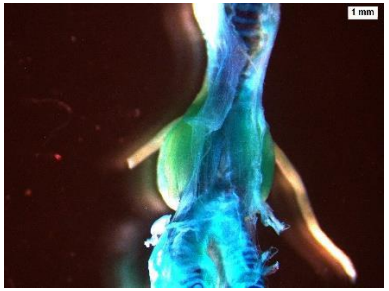
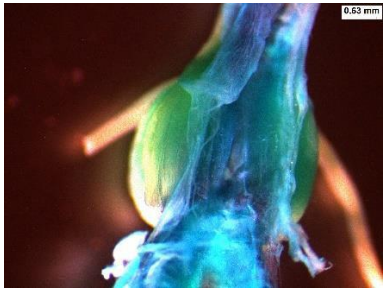
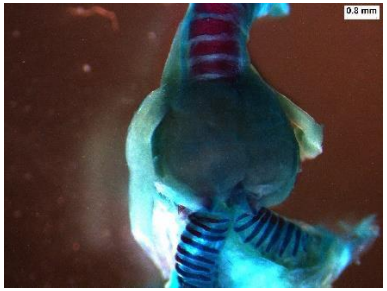
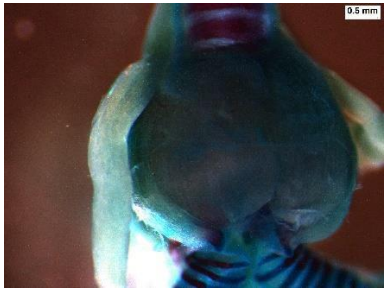
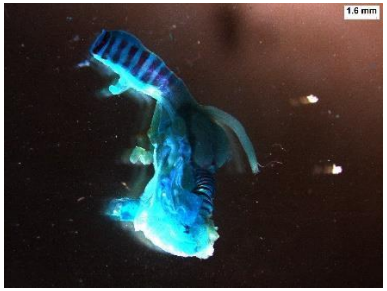
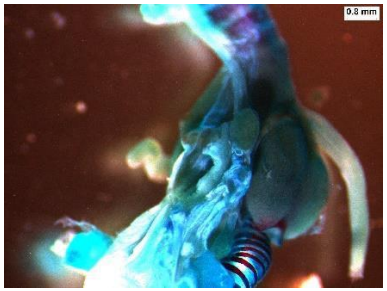
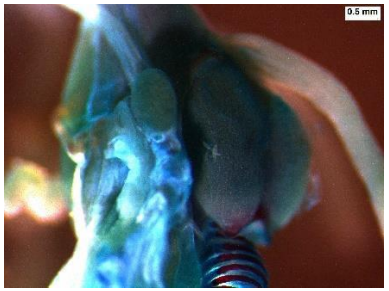


98

98

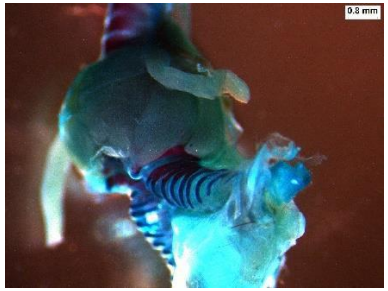
98



Eremopterix verticalis	98	98	98	98
				
Eremopterix leucotis (Appendix 2.C.4 a-c)	66a	66	66	66
				
	66	66	66	66
				

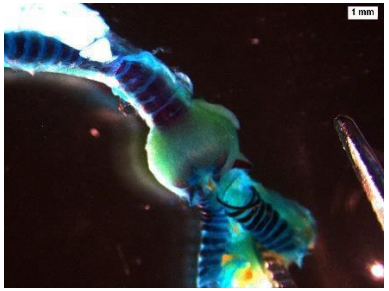
Eremopterix leucotis

66



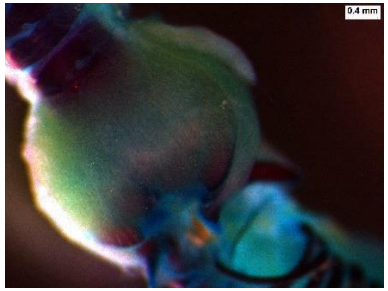
66

68b

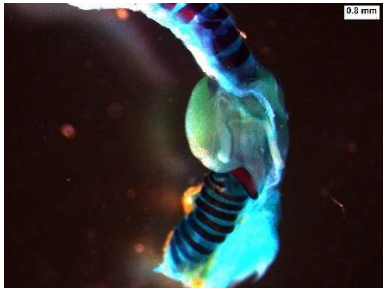


68

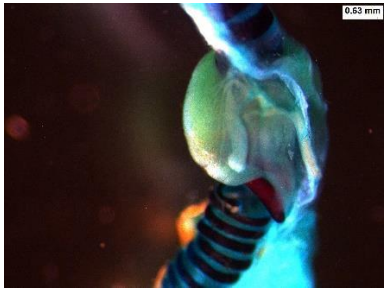
68



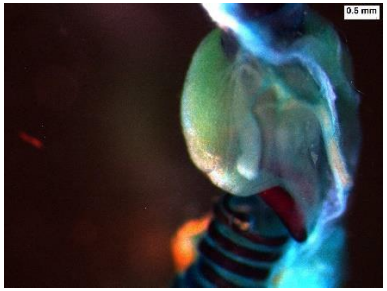
68



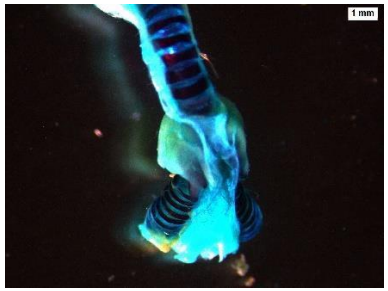
68



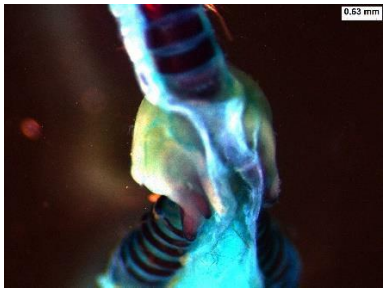
68



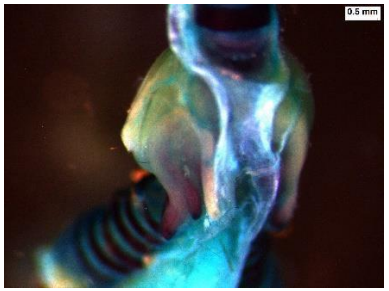
68



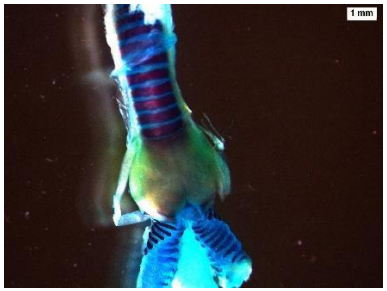
68



68

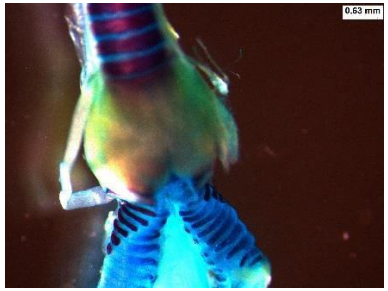


69c

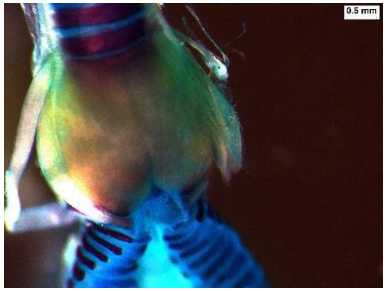


Eremopterix leucotis

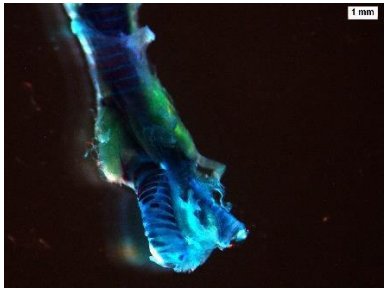
69



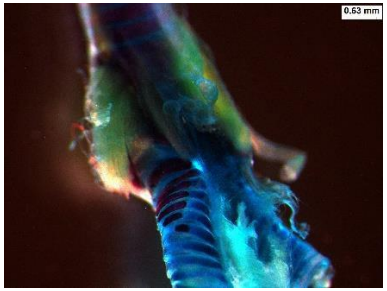
69



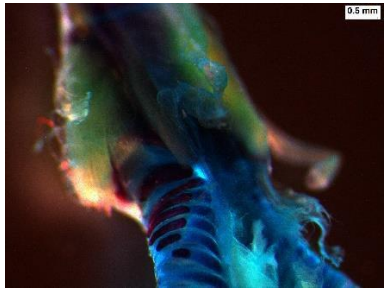
69



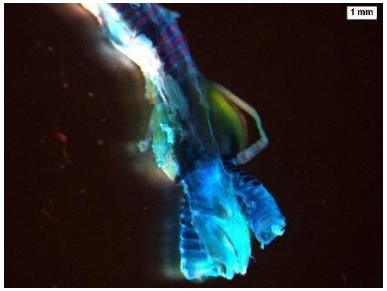
69



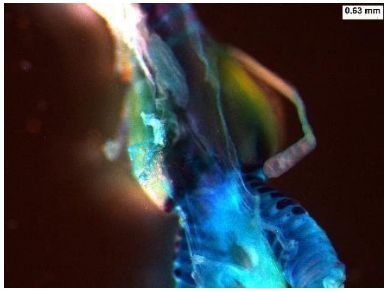
69



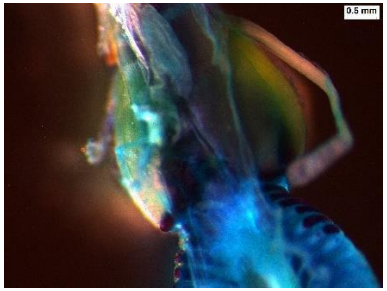
69



69

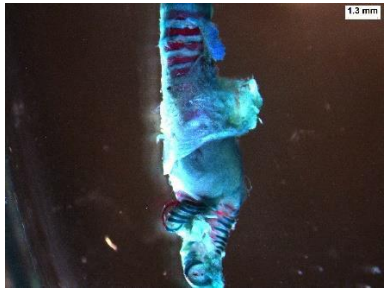


69

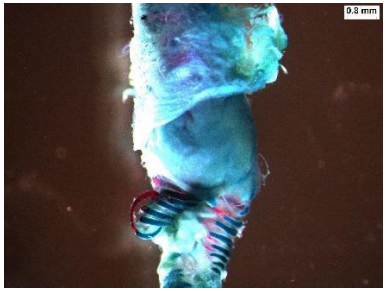


Chersomanes albofasciata (Appendix 2.C.5 a-c)

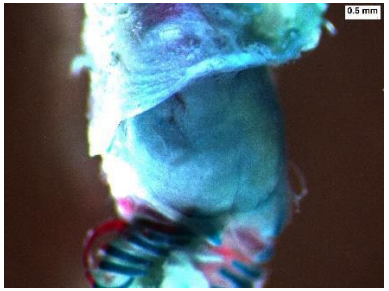
48a



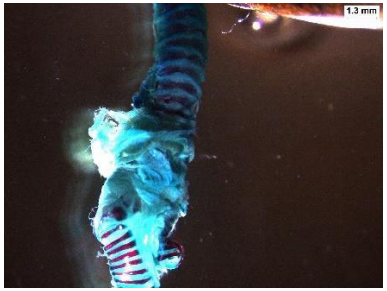
48



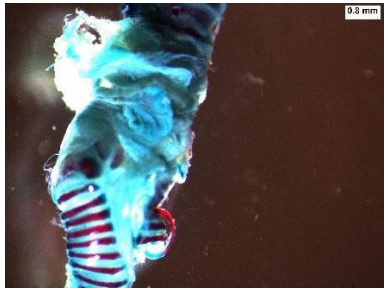
48



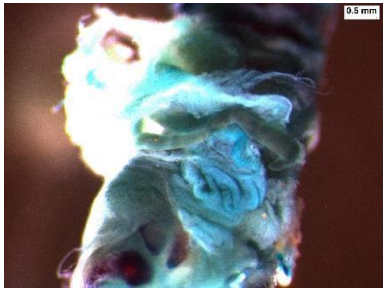
48



48

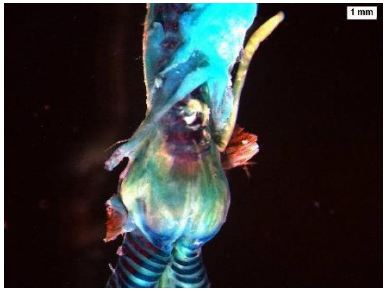


48

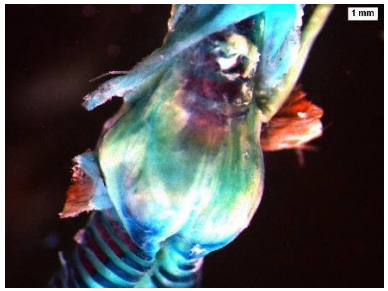


49b

49



49

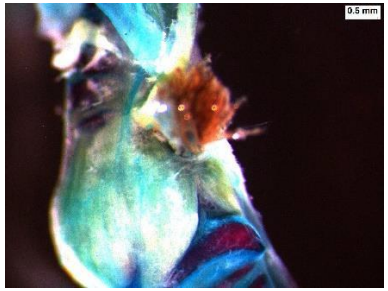
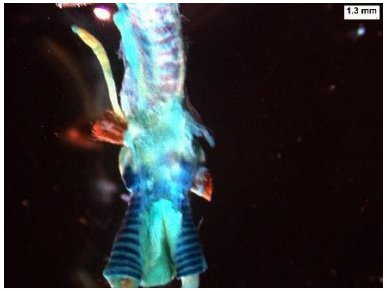
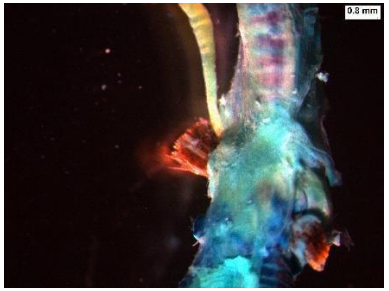
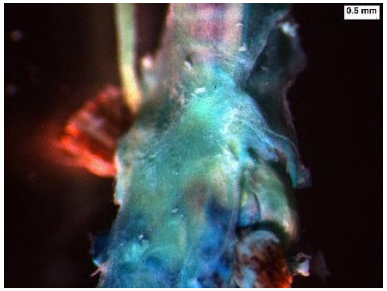
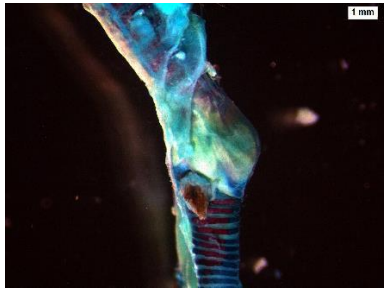
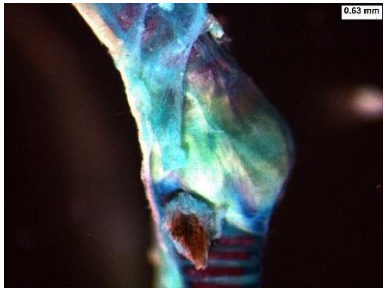
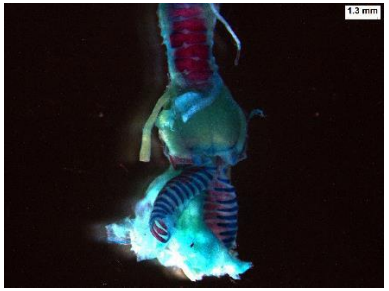
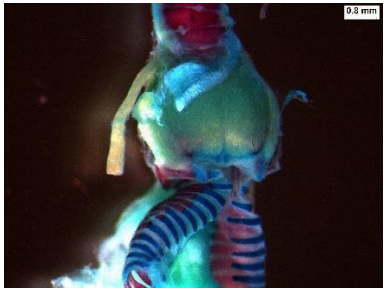
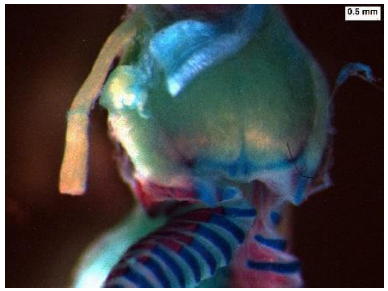
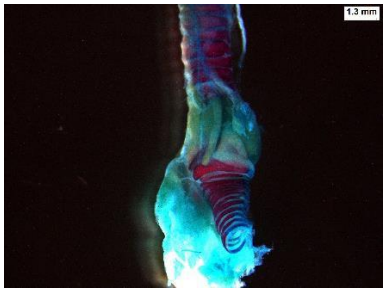
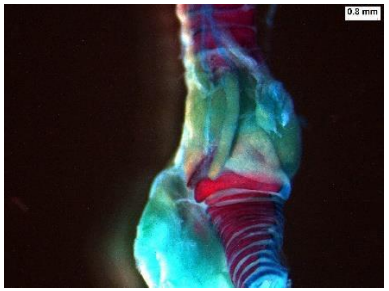
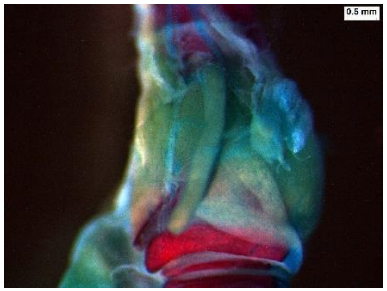


49

49

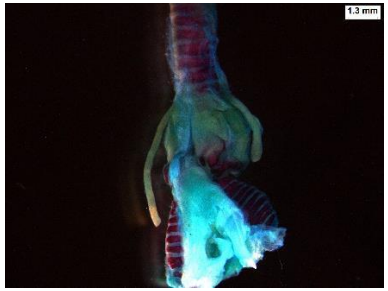
49

Chersomanes albofasciata

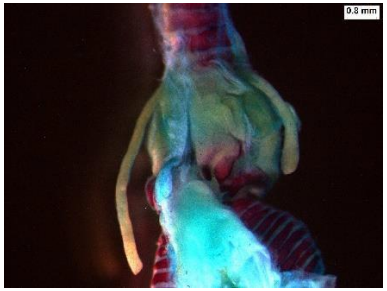
49	49	49	49
			
49	49	53c	53
			
53	53	53	53
			

Chersomanes albofasciata

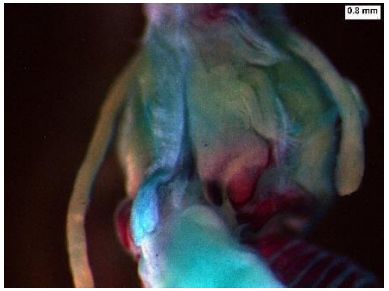
53



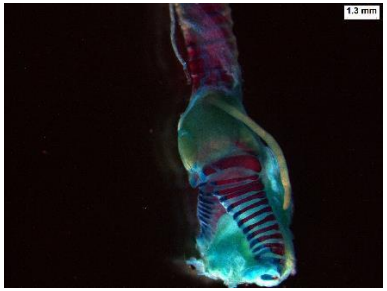
53



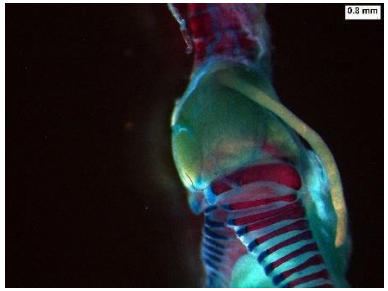
53



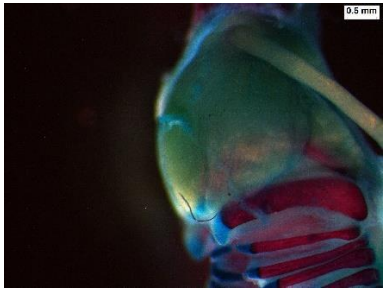
53



53



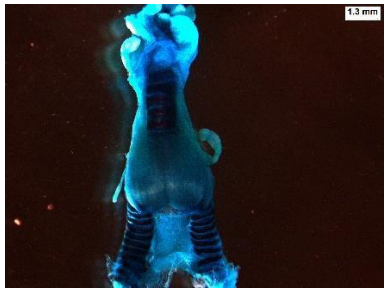
53



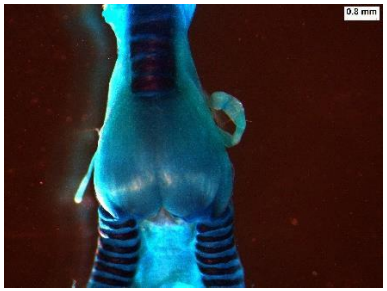
APPENDIX 2.4. GROSS MORPHOLOGY PLATES FOR OUTGROUPS.

Sylvietta rufescens (Appendix 2.O.1 a)

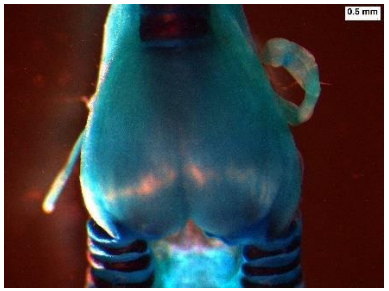
147a



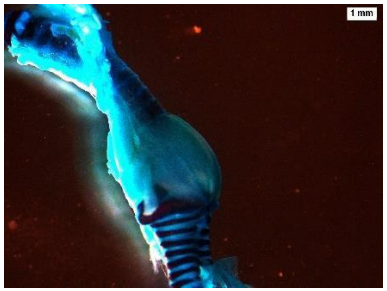
147



147

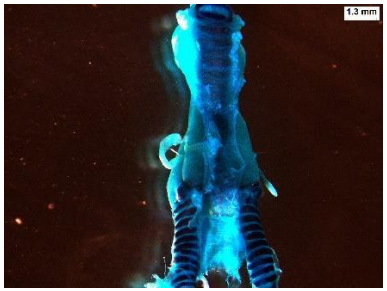


147

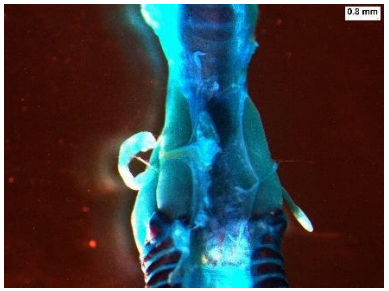


147

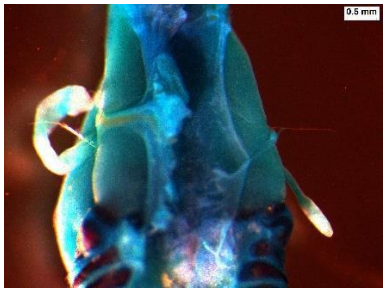
147



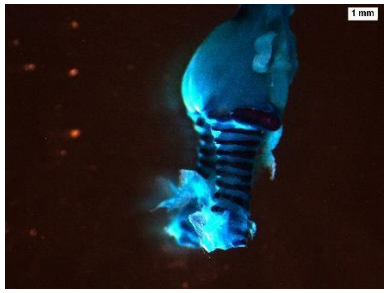
147



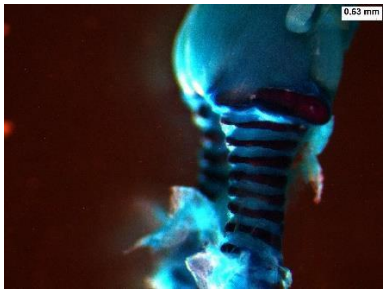
147



147

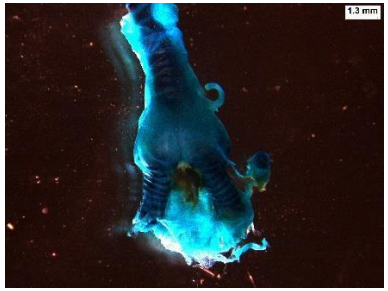


147

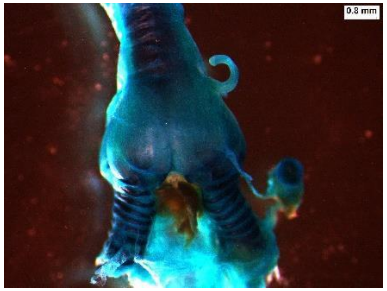


Sylvia borin (Appendix 2.O.2 a)

145a



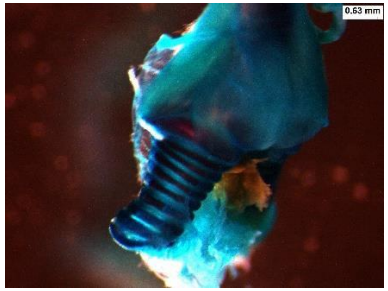
145



145

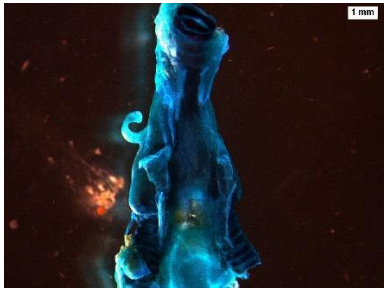
145

145

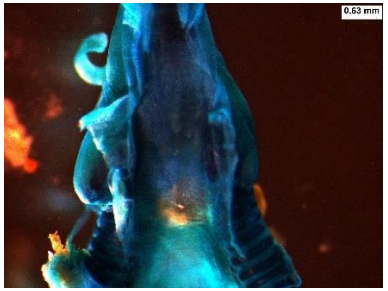


145

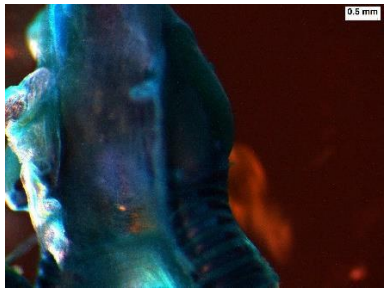
145



145

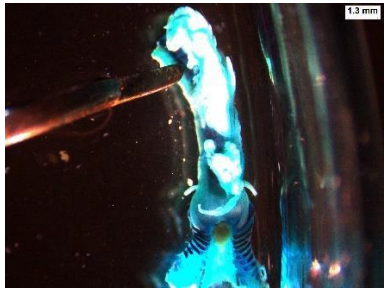


145



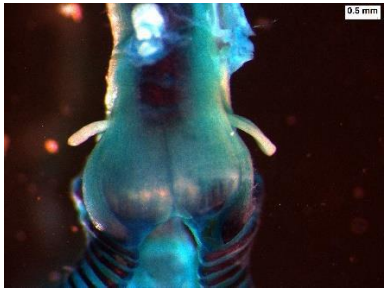
Hirundo abyssinica (Appendix 2.O.3 a)

148a

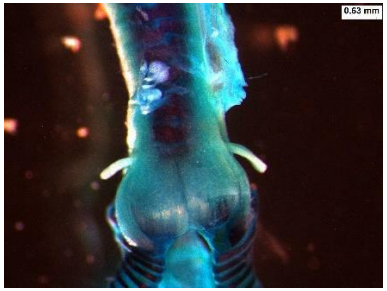


148

148



148

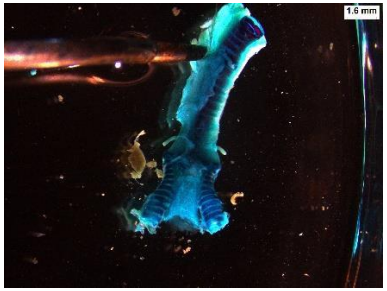


148

148

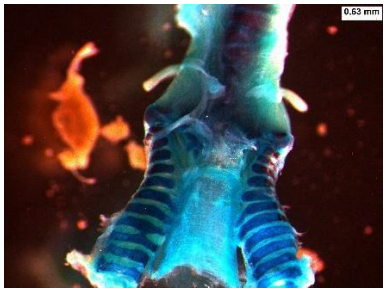
148

148

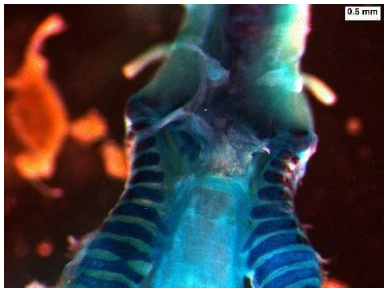


148

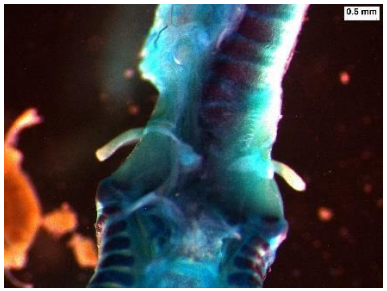
148



148

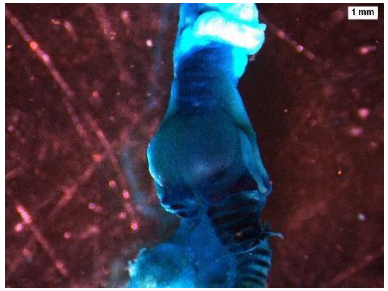


148

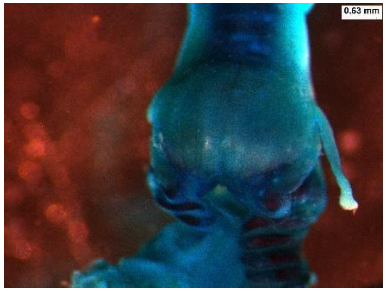


Cisticola chiniana (Appendix 2.O.4 a)

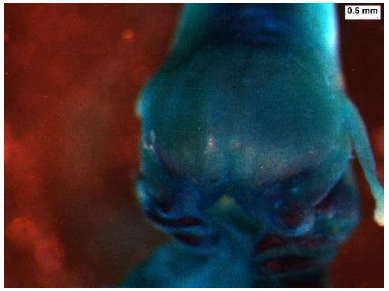
146a



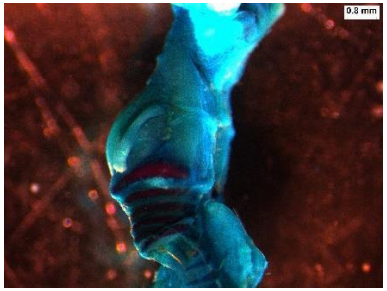
146



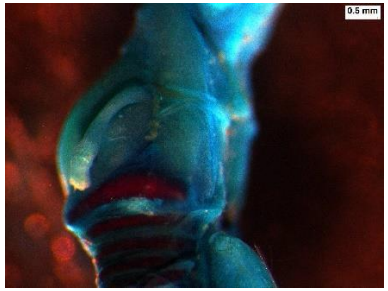
146



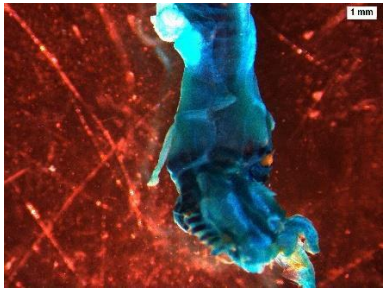
146



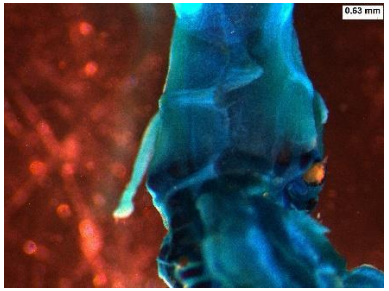
146



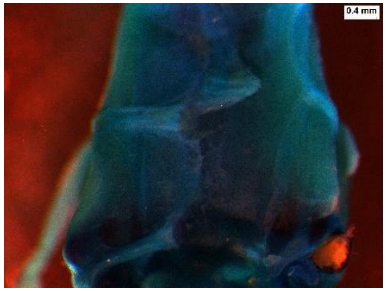
146



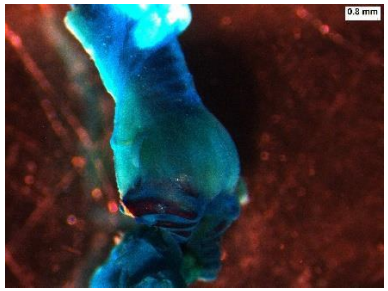
146



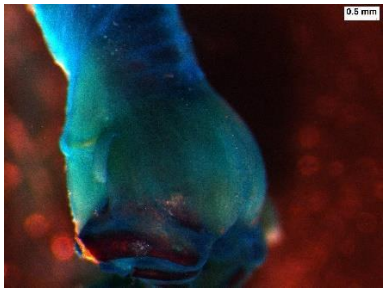
146



146

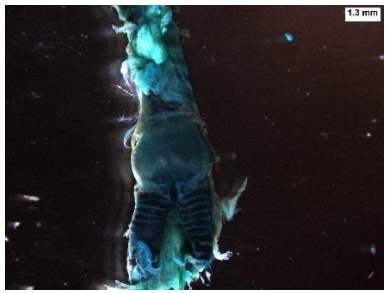


146

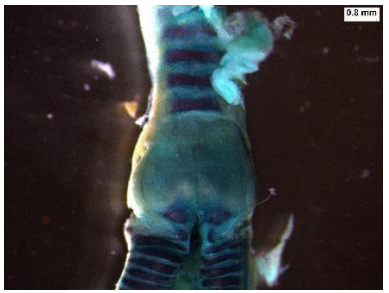


Anthus cinnamomeus (Appendix 2.O.5 a)

124a

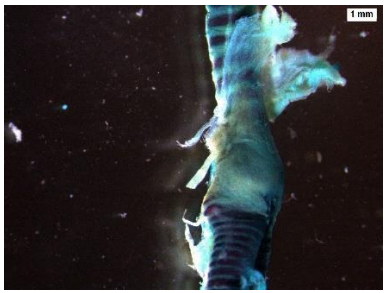


124

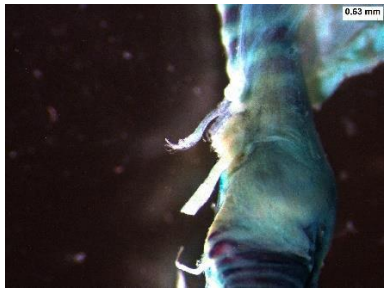


124

124

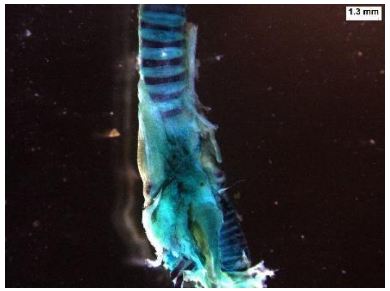


124

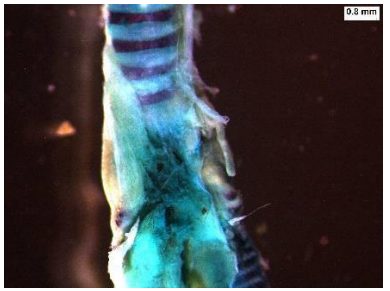


124

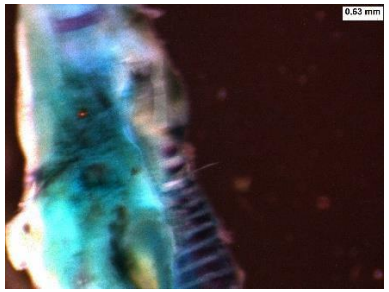
124



124

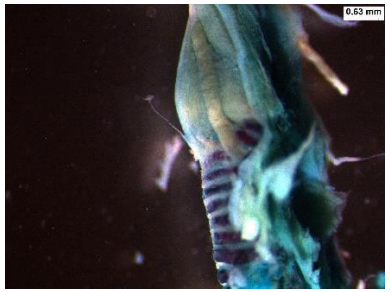


124



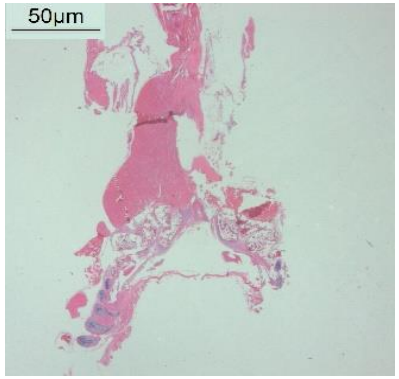
124

124

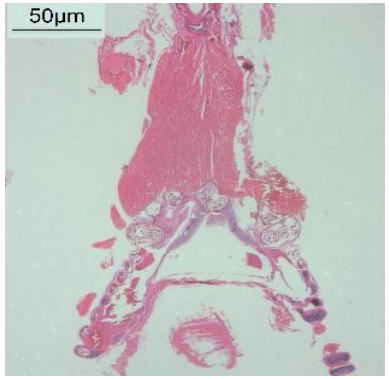


APPENDIX 2.5 HISTOLOGICAL SECTIONS OF SYRINGES OF DIFFERENT SPECIES OF LARKS AND OUTGROUPS SPECIES. FOR LABELLING, E.G. (APPENDIX 2.5.1 127A-C), 2 REFERS TO APPENDIX NUMBER; 5 – HISTOLOGICAL SECTIONS; 1 – A PARTICULAR SPECIES – GALERIDA MAGNIROSTRIS; 127 – SAMPLE NUMBER FOR GALERIDA MAGNIROSTRIS SYRINX, A – C A SERIES OF TISSUE SECTIONS. NB. ALL SECTIONS WITH NUMBER 127 COME FROM A SYRINX OF AN INDIVIDUAL BIRD AND 113 IS ANOTHER INDIVIDUAL BIRD BUT THE SAME SPECIES GALERIDA MAGNIROSTRIS WITH A-G SHOWING SERIES OF SECTIONS.

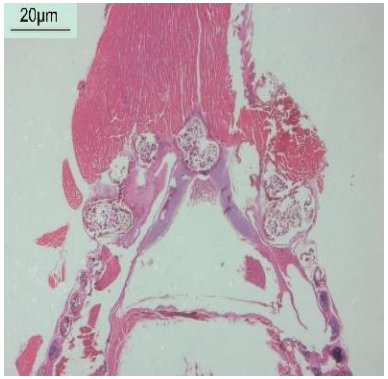
Galerida magnirostris (Appendix 2.5.1)



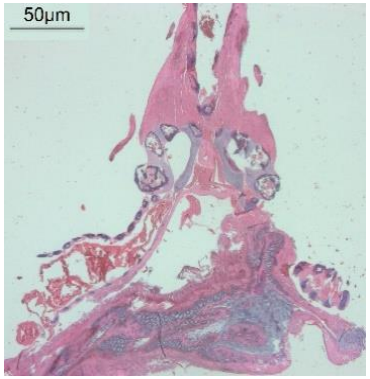
127a



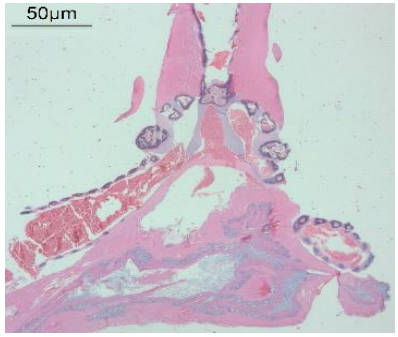
127b



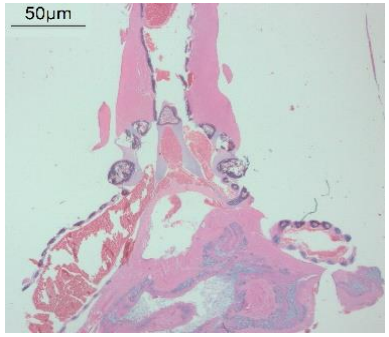
127c



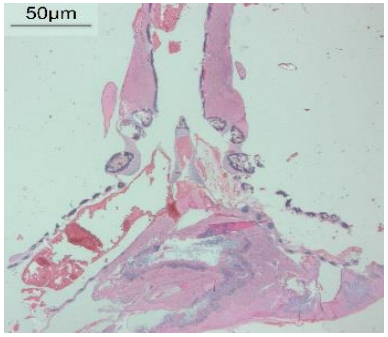
113a



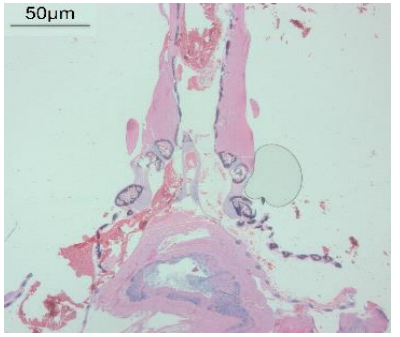
113b



113c

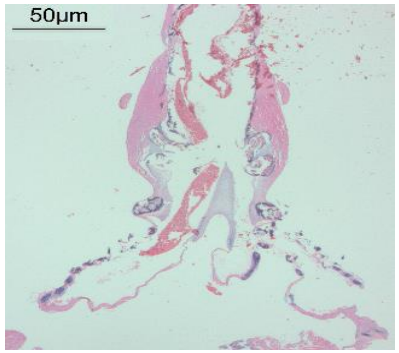


113d

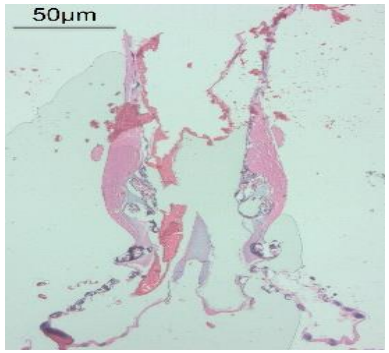


113e

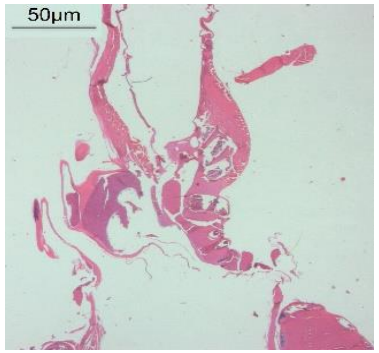
Mirafra fasciolata (Appendix 2.5.2)



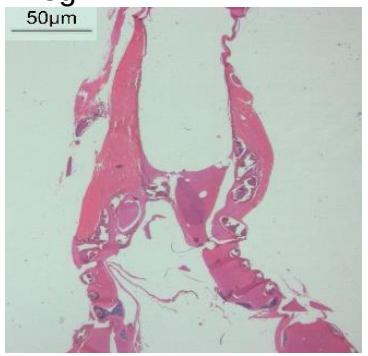
113f



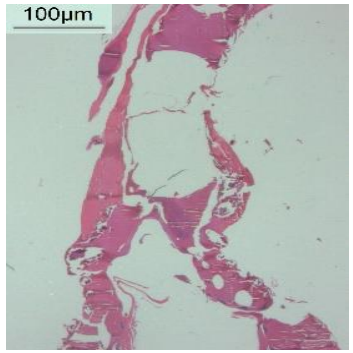
113g



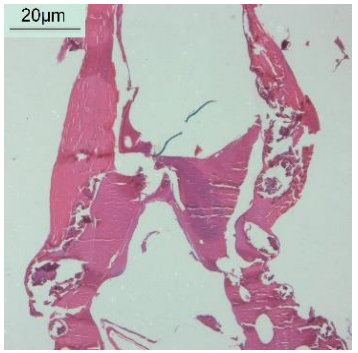
117a



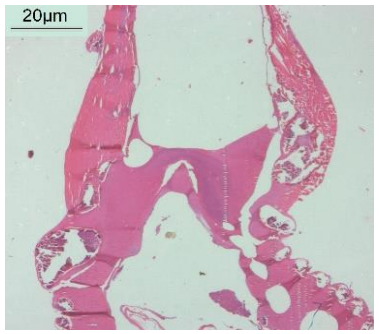
117b



117c

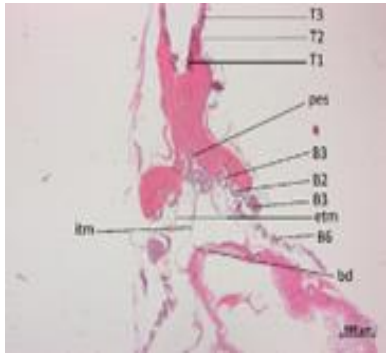


117d

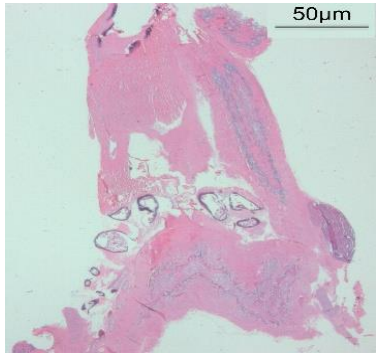


117e

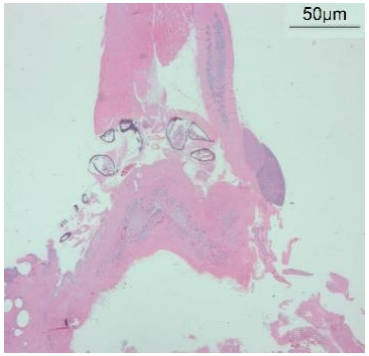
Mirafra cheniana (Appendix 2.5.3)



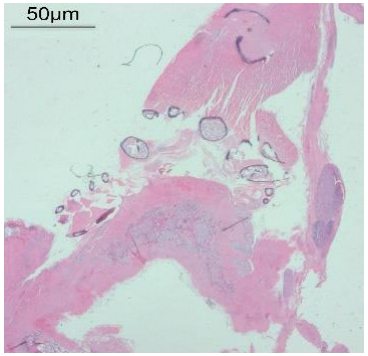
123a



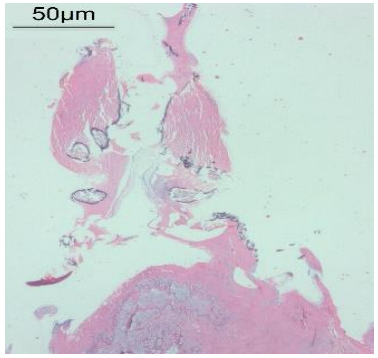
116a



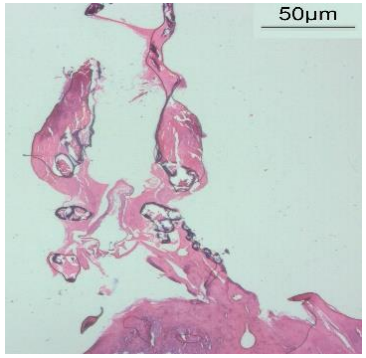
116b



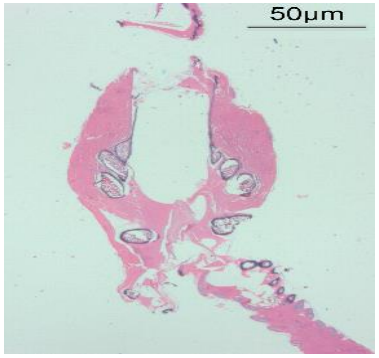
116c



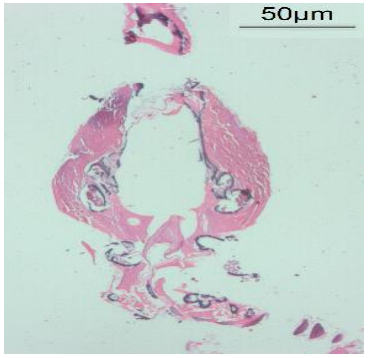
116d



116e

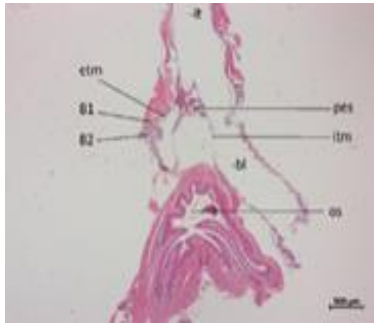


116f

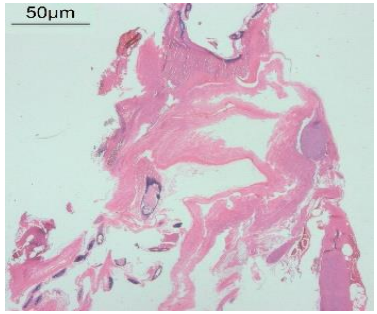


116g

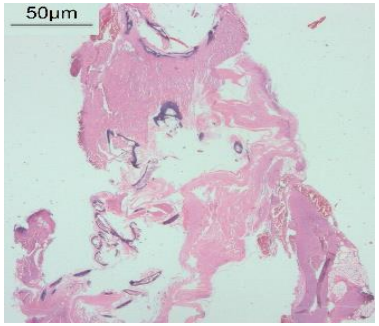
Mirafra passerina (Appendix 2.5.4)



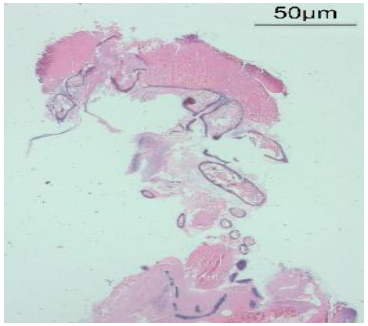
132a



133a

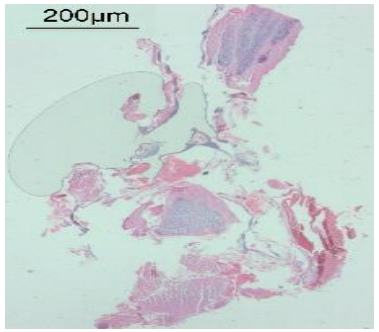


133b

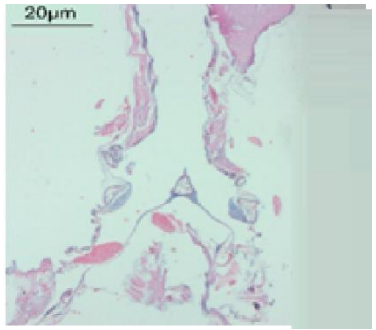


133c

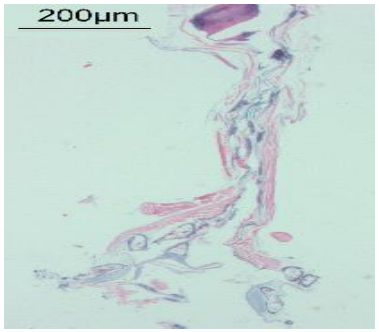
Acrocephalus palustris (Appendix 2.5.5)



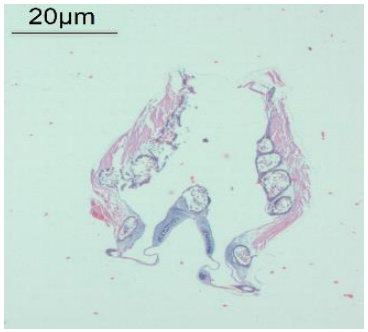
143a



143b



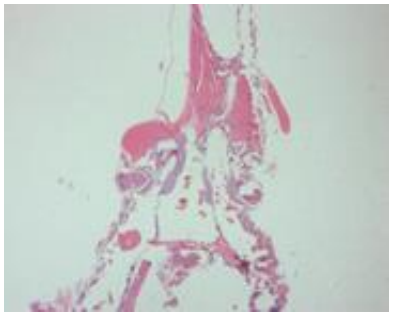
143c

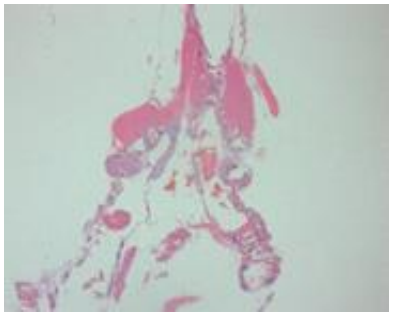


143d

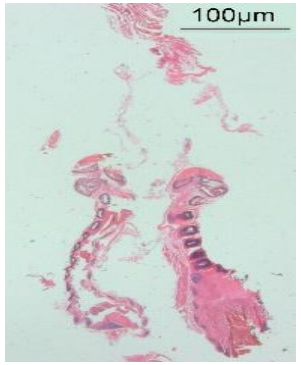

142a


142b


142c


142d

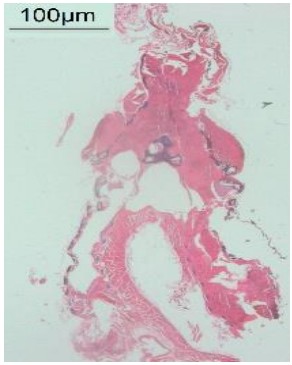
Anthus leucophrys (Appendix 2.5.6)



129a

129b

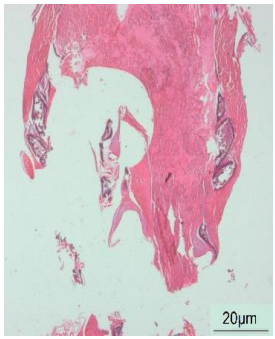
129c



129d

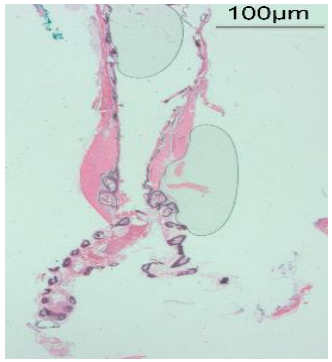
129e

129f

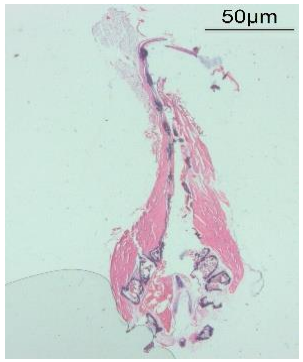


129g

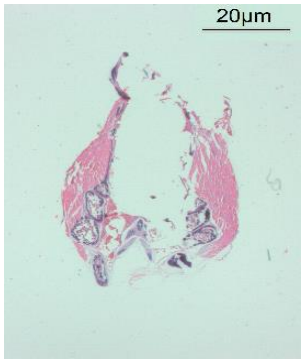
Anthus cinnamomeus (Appendix 2.5.7)



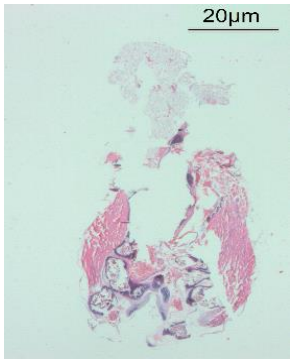
131a



131b

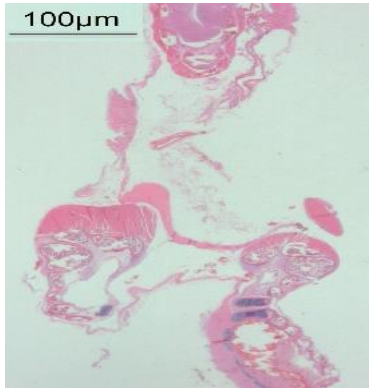


131c

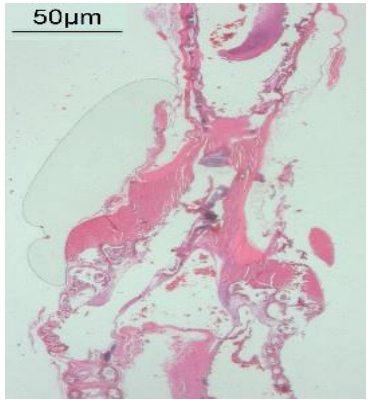


131d

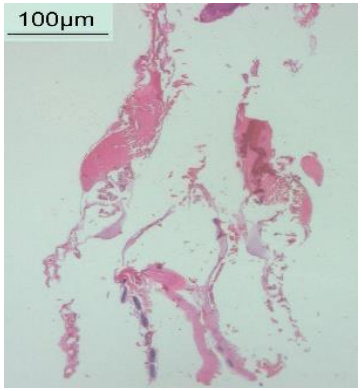
Macrorynx capensis (Appendix 2.5.8)



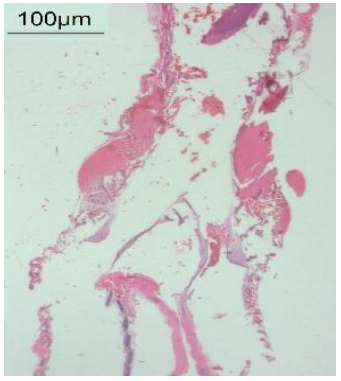
119a



119b

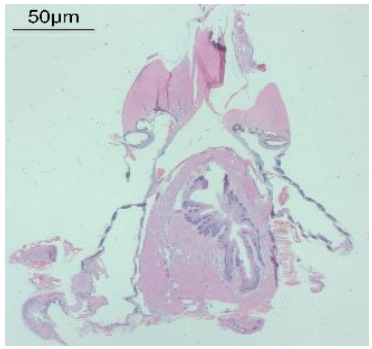


119c

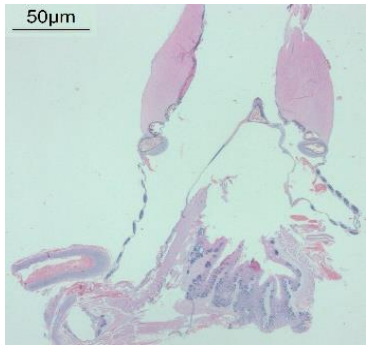


119d

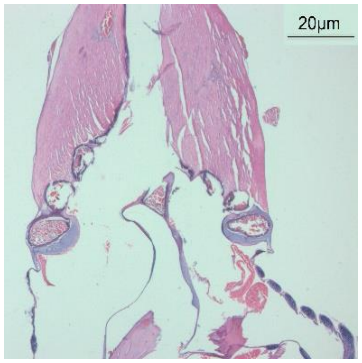
Cisticola chiniana (Appendix 2.5.9)



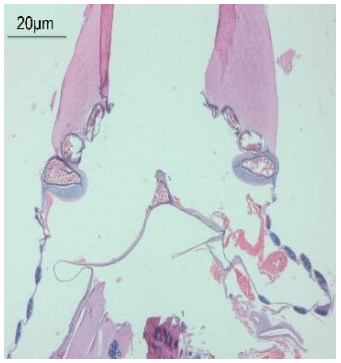
144a



144b



144c



144d

CHAPTER 3

The characterisation of larks (Passeriformes, Alaudidae) based on vocalisations

3.1 Background information

The class Aves has a global distribution and many birds are revered for their songs and the various sounds they produce. Without birds, the Earth would be a decidedly quieter planet as none of the other vertebrate taxa are as vocal, and relatively few invertebrate taxa produce audible sounds. Birds use their ability to fly to escape predation and reach suitable foraging and breeding grounds. Vocal communication is one of the most important phenomena in animal behaviour as it serves to pass on information from one individual to another during migration, territory defence, alarming and mate recognition (Naguib and Price 2013).

Intraspecifically, birds come into contact both vocally and through behavioural patterns when foraging, during courtship or a sexual context, territory claiming, alarming to potential predators, avoidance of threats and competitors; all requirements that ensure their prosperity and social affiliation (Kelley *et al.* 2008; Podos and Moseley 2010). The production of sound in birds can also be used to communicate over long distances or when it is difficult to see conspecifics such as in very dense vegetation, e.g. forests (Gill 1990). On the contrary, some birds such as storks and vultures barely use sound, instead, they use hisses and grunts to communicate instead.

The use of sound is most prominent among members of the order Passeriformes, or the ‘songbirds’, as they have a complex voice producing organ, the syrinx (Ames 1971). Sound production can also be influenced by several factors, including habitat, body size, sex, season, type of syrinx and morphology of the beak (Derryberry 2009; Podos *et al.* 2009). On another front, vocalisations were found to be delicate markers of speciation and population divergence (Miller and Baker 2009) and have been used in present-day species-

level systematics (Alström *et al.* 2007) and in clearing up the phylogenetic history of species groups (Mandiwana-Neudani *et al.* 2014).

Bird vocalisations are generally divided into songs and calls, but the clear distinction between the two requires scrutiny as there is no universal definition in the literature (Catchpole and Slater 2008). The term “song” is used to refer to long and complex, but sustained vocalisation mainly produced by males during the breeding season (Catchpole and Slater 2008), and is typically comprised of verses, syllables, phrases and trills (Bonnevie and Craig 2018). They are often rendered with specific, repeated patterns and are recognisable not only at the specific level, but often at the group and individual levels (Chen and Maher 2006). In contrast to a song, a ‘call’ tends to be shorter and simpler with sequences of phrases given by either sex throughout the year and serving mainly to alert and coordinate group behaviour (Catchpole and Slater 1995). Both song and call can be rendered individually, or through interaction between a male and female involved in duetting (Hall 2009).

3.1.1 Vocalisations and classification of taxa

Although the use of molecular data in phylogenetic studies contributes more to the systematics community, vocalisations have commonly been used in assigning the taxonomic rank or deduce phylogenetic relationships of non-Oscine birds compared to the rate at which it is used in Oscine birds (Isler *et al.* 1998). In birds, vocalisations that are not acquired through learning are considered phylogenetically informative (Miller and Baker 2009). The non-Oscines’ vocalisations are generally not learned, although evidence suggests limited learning in some Cotingidae (Saranathan *et al.* 2007), thus it is innate (Isler *et al.* 1998). Such vocalisations are found in 27 of 30 orders, excluding Passeriformes, Psittaciformes, and Apodiformes (Bradbury and Vehrencamp 1998; Jarvis 2006). The rate at which vocal divergence proliferates is much slower in non-Oscine birds and this may lead to higher phylogenetic components to their vocal signals (Seneviratne *et al.* 2012). On the other hand, Oscines are known to acquire their conspecific song through learning as well as incorporating songs or calls from other species (Lein 1978; Lanyon 1979; Weary *et al.* 1990) while the process of learning may lead to speciation, creating prezygotic separation through resident dialects (Baptista and Trail 1992; Edwards *et al.* 2005).

Avian systematists have generally shied away from using vocal characters of Oscines and this is explained by the difficulty that arises when attempting to distinguish between genetic and ecological components of vocalisations (McCracken and Sheldon 1997), and also the difficulty in detecting homology across taxa (Lanyon 1969). Vocal characters may be prone to convergent evolution due to the diverse selection pressure acting on these characters (McCracken and Sheldon 1997; Nicholls and Goldizen 2006). For example, lower and narrower frequencies in vocalisation are associated with bird species found in dense or closed vegetation compared to those inhabiting open habitats that tend to render vocalisation with broad frequencies (Morton 1975). This is because longer wavelengths propagate energy more proficiently through vegetation than the shorter wavelengths, which fade due to the scattering effects of vegetation. Furthermore, several repertoires of distinct song types can be found in numerous bird species from various clades, while other species have only one simple and stereotyped song or call (Price and Lanyon 2002; Lei *et al.* 2005). An interesting phenomenon occurs where species of birds learn song components from other species (heterospecific mimicry), making it even more challenging to distinguish homologous components from these learned songs (Lei *et al.* 2005). Alström and Mild (1993) used vocalisation to corroborate the relationship between two Oscine species, Berthelot's Pipit *Anthus berthelotii*, endemic to the Canary Islands and Madeira, and Tawny Pipit *A. campestris* found in central Palearctic from northwest Africa and Portugal to Central Siberia and on to Inner Mongolia. utilisation of songs of Oscines in phylogenetic studies have been criticised due to copying errors that may arise during a sensitive phase of learning (Thielcke 1970) and dialects across geographic ranges which can lead to separation of species (Rendall and Kaluthota 2013).

Suboscine songs are considered innate and can be indicative of evolutionary divergence between populations (Isler *et al.* 1998, Touchton *et al.* 2014), hence they are phylogenetically informative (Van Niekerk 2013). Raposo and Höfling (2003) have highlighted the challenges posed by the generalisation of findings such as the notion that Suboscine birds do not learn their vocalisations. To support this, Snow (1970) found that the young males of a Suboscine species, the Bearded Bellbird *Procnias averano* learn their songs from males. Therefore, the notion that Suboscines do not learn their songs or that their songs are inherited should be re-evaluated since this was based on a study of only

three species of Tyranids: Alder Flycatcher *Empidonax alnorum,* Willow Flycatcher *E. traillii* (Kroodsma 1984), Eastern Phoebe *Sayornis phoebe* (Kroodsma and Konishi 1991) and recently a study on Spotted Antbird *Hylophylax naevioides* (Touchton *et al.* 2014).

Although Oscines learn their songs, they are said to be evolutionary conservative (Irwin 2000; Päckert *et al.* 2003). Therefore, this could mean that they have a genetic basis, and similarities of vocal characters observed between species may be correlated to their phylogenetic relatedness. For example, the European warblers of the genus *Acrocephalus* (Catchpole 1980), Marsh Wren *Cistothorus palustris* (Kroodsma and Candy, 1985), oropendolas of the genus *Psarocolius* (Price and Lanyon 2002), goldcrests and kinglets (Regulidae: *Regulus*) (Päckert *et al.* 2003), Golden-spectacled Warblers, *Seicercus burkii* (Päckert *et al.* 2004) and shorebirds (Charadrii and Scolopaci) (Miller and Baker 2009).

Other researchers have pointed out that the difference between Oscine and Suboscine vocalisations is due to the organ that produces these vocalisations: the “syrinx” (Ames 1971; Tsukahara 2008). Oscine passerines have complex syringes with five to seven syringeal muscles and produce complex songs, while non-Oscines have as little as three tracheal muscles and have simple calls (Frank *et al.* 2007). Syringeal morphology has proven to be informative in many systematic studies of birds (Delacour and Mayr 1945; Humphrey 1955; Johnsgard 1961; Lanyon 1986; Livezey 1986; Prum and Lanyon 1989; Brown and Ward 1990; Prum 1992; Mobley and Prum 1995; Griffiths 1994a, b; Gaban–Lima and Höfling 2006; Zimmer *et al.* 2008; Mandiwana–Neudani *et al.* 2011). The vocalisations of Oscine passerines commonly incorporate mimicry of other birds related or distant species, and larks are arguably best known for this ability (de Juana *et al.* 2020; Kelley *et al.* 2008). In the family Alaudidae, mimicry of other birds is well-developed and heterospecific mimicry has been recorded in all three major lark clades identified by Alström *et al.* (2013), but is particularly well-developed in the genera *Melanocorypha, Calandrella* and *Mirafra*. Tsukahara *et al.* (2008) pointed out that birds that use mimicry in their songs cannot be phylogenetically classified in terms of vocalisation, unlike birds that do not incorporate mimicry because convergent evolution may affect the mimicked vocalisations. However, Payne (1986) stated that similarities in song quality may express genetic similarities regardless of whether the song is learned, and consequently may be employable in phylogenetic analyses. The author used the vocal characters of the Black-

throated Green Warbler *Setophaga virens* complex to reconstruct the phylogeny, and he concluded that the distribution of song traits among species indicates that cultural changes may have followed the same branching events as in the genetic differentiation of the species.

3.1.1.1 Study taxa

In this study the focus was on the larks, family Alaudidae (de Juana *et al.* 2020). The Alaudidae is a cosmopolitan family comprised of 21 genera and 98 recognised species (de Juana *et al.* 2020; Gill and Donsker 2020) (Fig. 1.1). Larks are primarily Old World birds found in Africa, Europe, and Asia with one species' range extending to the New World (Horned Lark *Eremophila alpestris*) and *Mirafra javanica* found in Australia. They are small to medium-sized passerine birds with dull plumage and are regarded as some of the best songsters among songbirds. They have long legs and relatively large wings, ranging in size from 15 g (*Eremopterix* and *Spizocorys*) to 75 g (*Melanocorypha*).

Africa has been described as the "lark continent" with all 21 genera and 78 species present, followed by Europe with 13 genera and 36 species, and one genus each in Australasia and the New World (Fig. 1.1). The family's species reach their peak in the semi-arid and arid regions of the Old World. Their distribution is not uniform and five centres of endemism are recognised: i) the Saharo-Sindian region, ii) the Caspian-Mongolian region, iii) the Oriental region, iv) the north-east arid zone of Africa (Kenya, Ethiopia and Somalia) and v) the south-west arid zone of Africa (South Africa, Namibia and Botswana) (White 1961; Moreau 1966; Dean and Hockey 1989; Barnes 2007; Allsopp *et al.* 2014). They occur in the tropics and temperate regions preferring open habitats ranging from deserts, woodlands and to high altitude mountainous areas and are only absent from closed-canopy forest. The majority of larks are found in deserts, grasslands and savanna habitats with 40 to 800 mm rainfall per annum (Dean and Hockey 1989).

The family shows an interesting range of sexual dimorphism with most males averaging 20–25% larger compared to females and the genera *Eremopterix* and *Melanocorypha* are the only larks that display sexual dichromatism (de Juana *et al.* 2020). The relatively drab plumage of larks is undoubtedly an adaptation which allows them to avoid predators, especially in open terrestrial habitats. However, despite their drab

appearance, there exists considerable intraspecific variation of plumage patterns and colours.

These defining taxonomic features aside, larks are also noted for their extensive range of vocalisations. Aurally, most species have whistling songs ranging from simple to complex songs with trills, harmonics, and also produce mechanical sound, i.e. wing clapping (Perrins 2003; Ryan and Marshall 2005). Although the delineation of larks as a family has long been resolved, relationships of genera and species within the family has been problematic. As such, the number of genera and species within the family have been fluctuating over the years. Approximately 20-23 genera associated with Alaudidae have variously been presented by different researchers (Sinclair and Ryan 2003; Donald 2004; Hockey *et al.* 2005; Barnes 2007). Alström *et al.* (2013) produced the most comprehensive multi-locus phylogeny of the larks to date and is based on mitochondrial and nuclear markers, which covered almost 80% of described species but managed to cover all genera in the family (Fig. 1.2). What is very prominent from this study is the finding that the family is represented by three major clades which were named clade A (hereafter Alaudid, B - Mirafrid, and C – Ammomanid) (refer to Chapter 1 and Appendix 1.1).

Despite this, Alström *et al.* (2013) failed to resolve some parts of the topology of Alaudidae phylogeny with certain areas remaining a concern to those who have an interest in larks. The phylogenetic hypothesis is, for the most part, resolved and supported by data, although some areas of the phylogeny across the clades (prominently A2c, B1a, B2 and C1a) incorporate a few polytomies or ineffectively supported nodes (Fig. 1.3).

Some areas of uncertainties were as follows:

i. in clade A, the topologies of the nuclear marker Ornithine Decarboxylase (ODC) and mitochondrial marker Cytochrome b (Cytb) trees differed from each other, which resulted in three strongly supported incongruent topologies.
ii. the two monotypic genera *Chersophilus* and *Eremalauda* (A1b) and complex (A1a) were strongly supported by data. The *Calandrella cinerea–brachydactyla–acutirostris* complex (A1d) and *Eremophila* (A1e) sister relationship was equally surprising.

iii. the data was inconclusive with respect to the relationships among the three species of *Alauda*, although MLBS (72%) and PBS (67%) suggest that *A. arvensis* and *A. gulgula* are sisters.

iv. five *Spizocorys* species (A2c) and the Short-tailed Lark *Pseudalaemon fremantlii* were strongly supported, although for half of these only Cytb and 16S were available. *Pseudalaemon fremantlii* was subsequently moved to the genus *Spizocorys*.

v. in clade B, clades B2a and B2b were both firmly supported. However, Cytb and 16S were accessible for everything except one of these species, albeit most of the relationships inside clade B2a aside from the sister connection between *Calendulauda barlowi* and *C. erythrochlamys* are not fully resolved. In clade B1a, the relationship of the five Asian taxa was unresolved wherein 16S sequences were unavailable.

vi. clades C1 and C2 are both strongly supported by the data. Their sister relationship seems strong (SLAs: 16S PP: 0.94, myo PP: 0.92, RAG PP: 1.00), though it was strongly contradicted by ODC, according to which clade C1 was part of clade A + B (PP: 0.99). Within C1a, a clade containing five species of *Eremopterix* is well-supported, although the relationship among these is effectively uncertain.

Generally, the phylogeny produced in Alström *et al.* (2013) featured most relationships as revealed by the molecular data but with some parts being incongruent with the past morphology-based characterisations.

3.1.1.2 Data types used to characterise larks

Traditionally, the designation of lark genera was based on morphological characters. The distinctive morphological features of larks with regard to the tarsus and syrinx, is sufficient to distinguish members of the family. Generally, it is understood that classifications that are dependent on morphology alone show less variation and may even be susceptible to convergent evolution masking specific and generic boundaries (Leisler *et al.* 1997). Nevertheless, their distinctive morphological features with regard to the tarsus and syrinx described above, is sufficient to distinguish members of the family and there are no lark

genera that have been previously placed in other families (Winterbottom 1962). However, morphological features within the family are either excessively flexible or too plastic to be used in designating the genera, therefore, defining the genera in the family have been problematic (Winterbottom 1962).

Meinertzhagen (1951) utilised habitat preference, the length and shape of the hind claw, plumage colour and pattern, and bill shape in one of the most cited efforts to infer phylogenetic relationships in the family. The outcome of that study found that most species were classified in genus *Mirafra*. Maclean (1969) contended that the characters that Meinertzhagen (1951) used were too variable and susceptible to convergent evolution. The generic revision by Maclean (1969) included cranial structure, nest structure, and behavioural features, and he restricted the revision only to the larks he was familiar with. The practice of classifying larks using these traditional taxonomic rankings, has seen the number of genera and their structure vary drastically over the years (e.g. Roberts 1940; Meinertzhagen 1951; Vaurie 1951; Macdonald 1952a, b, 1953; Verheyen 1958; Peters 1960; Clancey 1966; Harrison 1966; Maclean 1969; Wolters 1979; Clancey 1980; Dean *et al.* 1992; Pätzold 2003; Dickinson 2003; de Juana *et al.* 2020). Prior to Alström's *et al.* (2013) study, only one article on the molecular phylogeny of larks based on mitochondrial sequences had been published and this research had the majority of African species. The data offer a baseline for a reassessment of lark relationships and classification, as well as the establishment for remarks on the morphological development in this bird family.

3.1.2 Aim

The aim of this chapter was to characterise the species of larks (Alaudidae) based on their vocalisations and use song parameters to assess the species affinities.

3.1.3 Objectives

The objectives were to:

i) statistically assess the distinctiveness of the three major clades (A - the Alaudid, B – the Mirafrid, C – the Ammomanid) recovered in Alström *et al.* (2013).
ii) statistically predict the group membership of taxa within clades and at genus level.
iii) produce the comprehensive descriptions of the songs of larks.

3.2 Materials and methods

3.2.1 Data collection

3.2.1.1 Sampling coverage

The focus in this chapter was on the southern African lark species. However, to be able to put the findings based on vocal analysis within a realistic context and to be able to draw informed conclusions, vocalisations of some larks from elsewhere in Africa and other parts of the Alaudidae range were also included in the analysis. The inclusion of species found in other regions was possible due to the availability of online sound libraries: Cornell University's Macaulay Library of Natural Sounds (MLNS), Avian Vocalization Centre (AvoCet - https://avocet.integrativebiology.natsci.msu.edu/), Xeno-canto (http://www.xeno-canto.org/) (Table 3.1) and individual ornithologists.

3.2.1.2 Gathering of sound recordings

Field recordings were mostly obtained during the breeding seasons of the targeted species to increase the likelihood of recording singing birds. Data were collected mainly during spring/summer (October to November 2016) in Free-state Province, mid-summer to late autumn/early winter (March to June 2017) in Limpopo, Mpumalanga and from August to December 2017 in the Northern and Western Cape provinces. Recordings were obtained in the field using either a Sony TCM-5000, Sony TC-D5 Pro II or a Marantz PMD 661 MK iii Recorders and a Sennheiser ME-80, MKH-70 or ME-67 shotgun microphones.

In this chapter, song was defined as simple or complex, musical or harsh and short or long vocalisation, mainly produced by males during the breeding season (Catchpole and Slater 1995), composed of strophes or verses and used primarily in mate attraction or retention and territorial claiming contexts

(https://avocet.integrativebiology.natsci.msu.edu/avocet/types_of_bird_sounds).

Contrary to the descriptions and visual representations of songs of larks in literature, which may largely be based on single recordings from one individual bird, this study analysed song strophes from multiple recordings from different individual birds representing

the respective species. Some species are as per the records in literature also known to mimic songs of other species and hence this justifies the reason to consider multiple recordings from different individual birds per species. This was to allow comparison and consideration of good quality song strophes while eliminating song strophes that have mimicked parts from other species if encountered. Song recordings assembled included those collected in this study and those analysed in Alström *et al. (*2013). Additional recordings were sourced from sound library archives, including Cornell University's Macaulay Library of Natural Sounds (MLNS), Avian Vocalization Centre (AvoCet), Xeno-canto (http://www.xeno-canto.org/) and various individuals with private collections of lark recordings.

3.2.2 Data analyses

3.2.2.1 Generation of spectrograms

Songs were imported in GoldWave, Inc. version 5.70 (GoldWave Inc 1993) for the trimming and selection of good quality parts called strophes. Strophes are defined as a unit of a song that gets repeated a few or a number of times; and are separated by a pauses and consists of elements that are also separated by a pauses.

Guidance towards the selection of the actual song strophes of particular species was sought in field guides and ornithology handbooks. The selected strophes were then analysed using Avisoft SASLab Pro Software Version 5.2.11 (Specht 2017). To generate spectrograms (visual representations of sound), the analysis performed followed a Fast Fourier Transform (FFT) with a sampling frequency of 22 050 Hz, FFT-length of 1024 points, the frequency resolution of 60 Hz, Frame: 100% and Bartlett Window Function. Avisoft analyses individual elements (peculiar, vocalised event/note), or a continuous line or band on a spectrogram in a strophe and this means that the same variables were analysed for each element.

From the song strophes that were selected to serve as a reference representing various species, the following strophe variables were examined for the species analysed: strophe duration (denoted as S_{dur}: the difference between the end time of the last element and start time of each element in a strophe), minimum frequency (denoted as F_{min}: the

lowest frequency in a strophe), maximum frequency (denoted as F_{max}: highest frequency in a strophe), frequency bandwidth (denoted as F_{band}: the difference between F_{max} and F_{min} in a strophe), peak frequency (F_{peak}: the highest energy in a strophe) and the number of elements (denoted as N_{ele}: all the elements in a strophe). The parameters were as follows: F_{min}, F_{max}, F_{band}, F_{peak}, S_{dur} and N_{ele} (Fig. 3.1a). It is worth noting that Avisoft generates data for each element across the strophe and in this context, values considered for each variable represented a record across the strophe. To work out F_{min}, lowest frequency across all the elements of the strophe was considered. The same method was applied for F_{max}, the highest frequency across the strophe was considered. For F_{band}, the difference between the element having the highest frequency and the element having the lowest frequency was computed. The peak frequency (F_{peak}) was selected from the element having the highest peak frequency across the strophe. S_{dur} was computed as the difference between the end time of the last element in the strophe and start time of the first element in the strophe and, lastly, N_{ele} refers to the number of individual elements in the strophe. It should be noted that some strophes have for example, trilling and warbling parts in their strophes rendered with very short intervals. They were recorded as grouped elements, therefore, considered a single element (see annotations in Fig. 3.1a).

3.2.2.2 Statistical analyses

The dataset which consisted of six strophe variables was run through statistical analyses that were performed in R statistical software (R Development Core Team 2018). However, Past3 (Hammer 2005) was used for better visualisation of the biplots. The variables did not fit normality in the distribution of frequencies and therefore, were Log_{10}-transformed by applying the logarithmic transformation.

Univariate and multivariate methods were used to analyse the variable data. Firstly, each variable was tested using one-way Analysis of Variance (ANOVA) to determine if there was significant difference looking at the level between or within the clades. The variables that showed significant difference were then chosen for further analyses. Two multivariate methods, Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) were conducted on the variables that were correlating to one another. Principal Component Analysis was considered for reducing the number of variables from

those originally measured to a small number of reciprocally independent ones henceforth, denoted to as principal components (PC). Ideally, the extracted principal components should at best explain most of the variation comprised in the original dataset (García *et al.* 2014). Discriminant Function Analysis was considered for its ability to determine group membership (Titus *et al.* 1984).

In PCA, the component scores contributing more than 70% of the data were extracted for each data point and used for further analysis (García *et al.* 2014). To test for significant differences in song strophes between and within clades, a nested multiple analysis of variance (MANOVA) with all the principal component (PC) scores was performed. This was conducted as part of DFA to observe if there was significant difference in the PC scores generated. Discriminant Function Analysis was conducted to predict and validate the group membership of song strophes of species in accordance with the circumscribed clades (A – the Alaudid, B – Mirafrid, C - Ammomanid) based on the relationship between the three PC scores (Blumstein and Munos 2005; Podos 2007). Partition plots were prepared for all variables identified through the DFA. To examine which parameters led to differences in strophes between and within clades, a nested ANOVA for each of the three PC scores was conducted separately and *Post-hoc* Turkey's honestly significant difference (HSD) test was used to examine the effect details. The contribution of the different strophe parameters to the discriminant model was also examined. The above-mentioned statistical steps were conducted at a genus-level to evaluate which of genera contributed more to the variation across each of the clades. Finally, within each clade, all the aforementioned statistical analyses were similarly performed at a clade level.

3.2.2.3 Description of song strophes

Description of song strophes was generated visually from spectrograms (spanning both quantitative and qualitative variables) and aurally across the study species. The quantitative characteristics were as follows: F_{min}, F_{max}, F_{band}, F_{peak}, S_{dur} and N_{ele}. The following qualitative variables were used to describe the songs: strophe length (short, intermediate, long), general strophe pitch (descending, ascending, stable), strophe type (aurally: musical, predominantly harsh), grouped element-ending (absent, present), grouped element-ending structure (not applicable, warbling/bubbling, trilling), wing

clapping, wing clapping incorporation in strophe (not applicable - applies to the species where wing clapping is non-existent, absent - refers to instances where wing clappings are independently inserted not interfering with the sound, present - refers to instances where wing clappings are inserted in a way which make them form part of the sound either rendered independent of the song strophe or slightly running into the song strophe), mimicry (sourced from literature: unknown/not recorded, known/recorded) (Table 3.2).

3.3 Results

3.3.1 Univariate findings – one-way ANOVA

The outcomes of univariate analysis by mean of one-way ANOVA returned only three variables that were found to differ significantly ($P < 0.05$) between clades: maximum frequency (F_{max}, $P = 0.05$), peak frequency (F_{peak}, $P = 0.05$) and frequency bandwidth (F_{band}, $P = 0.01$) (Table 3.3). *Post-hoc* Tukey's Honest Significant Difference (HSD) test for F_{max} and F_{peak} showed that clades A and C differed significantly ($P < 0.05$), while F_{band} showed that clades A and C differed highly significantly ($P < 0.01$). There was no significant difference between clade B and clade A and clade B and clade C (Fig 3.2). Clade B largely overlapped with clade A (Fig. 3.2).

3.3.2 Multivariate findings - Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA)

The three variables F_{min}, S_{dur} and N_{ele} were excluded from PCA and DFA since they were found not to be statistically significantly different. Principal component analysis extracted only one component with an eigenvalue of > 1.0, explaining 72% towards total percentage variance (Table 3.4), while component two had an eigenvalue = 0.8366 which contributed 23% of the total percentage variance. Both components combined explained 95% of the total percentage variance across the clades (Table 3.4). To determine the eigenvalues to choose as they correspond to principal components for further analysis, this is driven by the preferred percentage of variance suitable in explaining the variation in the data (Jollife 2002; Peres-Neto *et al.* 2005). Approximately 80% of the variance in data is sufficient in explaining the data, therefore, in this case, two PC scores best explain the variation in the

data. Further to this, the Scree Plot shows PCs and their corresponding eigenvalues (Fig. 3.3). F_{max}, F_{peak} and F_{band} had positive loadings on PC1. Only loadings exceeding 0.50 were reported (Table 3.4). On the other hand, PC2 had negative loadings of F_{max} and F_{peak} and positive loading of F_{band} (Fig. 3.4). There were significant differences between the clades (Table 3.5) using PC1 (one-way ANOVA; PC1: $F_{1.90} = 6.309$, $P < 0.01$). When the loading on a variable is positive on a Principal component (PC), it implies that individuals that are within the influence of such a component will have the highest measure of the variable, e.g. positive loading of F_{band} on PC2 (Fig. 3.4) pulled *Certhilauda subcoronata "*C.sub", which implies that *Certhilauda subcoronata* (clade C) has the highest frequency bandwidth and positive loading on F_{peak} on PC1 pulled *Mirafra affinis "*Baff", which implies *Mirafra affinis* (clade B) has the highest peak frequency. When the loading on a variable is positive on a Principal component (PC), it implies that individuals that are within the influence of such a component will have the highest measure of the variable. For example, loading of F_{band} on PC2 (Fig. 3.4) pulled *Certhilauda subcoronata "*C.sub", which implies that *Certhilauda subcoronata* (clade C) has the highest frequency bandwidth. The negative loading on F_{band} on PC2 pulled *Mirafra affinis "*Baff", which implies *Mirafra affinis* (clade B) has the lowest bandwidth frequency. Song strophes from all three sampled clades (clade A, B, C) differed significantly from one another (MANOVA: $F_{4,546} = 2.22$, $P < 0.05$).

Based on the DFA, the strophes that were classified correctly to their clades accounted for 55% of those analysed (Table 3.6). As far as the partition plot (Fig. 3.5) is concerned, the blue-shaded area represents a cluster for clade A, the white-shaded area is for clade B cluster while the pink-shaded area is for clade C cluster. Taxa are shown by alphabets A, B and C and if they are classified in the clade they do not belong to, the alphabets will be red. The partition plot only serves to tell which taxa are correctly or incorrectly classified or grouped without identifying the species. Among the 27 species that were analysed in clade A, 19 species (70%) were classified correctly, seven species were classified in clade B and one species was classified in clade C. There were 25 species analysed in clade B, 11 species were classified in clade A, 10 (40%) were classified correctly in clade B and four species were classified in clade C. Finally, clade C had 17 species analysed of which five were classified in clade A, three species were classified in clade B and nine species (53%) were classified correctly in clade C. These classifications

are clearly mapped in the Partition plot (Fig. 3.5) where species in clade A and B overlapped largely with a few species belonging to clade B being misclassified in clade C. There is a clear separation between member species of clade C from those belonging to clade A and B even though with some clade C taxa having been misclassified in clade A and B. Table 3.6 shows how these taxa were grouped or classified.

3.3.3 What do song strophes tell about the grouping of species within each clade and across genera?

The six variables analysed in this chapter influenced the species differently within each clade. Therefore, each clade was analysed separately to determine which of the variables contributed to the variance within these clades.

Clade *A* – the Alaudid species

Within clade A, one-way ANOVA returned four variables, maximum frequency (F_{max}), peak frequency (F_{peak}), number of elements (N_{ele}) and strophe duration (S_{dur}) that differed significantly ($P < 0.05$) within clade A (Table 3.7). *Post-hoc* Tukey's HSD test for the four variables; F_{max}, F_{peak}, N_{ele} and S_{dur} conducted in the ANOVA test is presented in Table 3.8. *Post-hoc* Tukey's HSD test on F_{max} showed that *Galerida* differed significantly at $P < 0.01$ from *Alaudala* and *Melanocorypha*, *Spizocorys* differed significantly at $P < 0.05$ from *Alaudala* on F_{peak} and N_{ele} per strophe, *Lullula* differed significantly at $P < 0.05$ from *Spizocorys* on S_{dur}.

Principal Component Analysis extracted only one principal component with an eigenvalue of >1.0 explaining 58% of the total % variance, also demonstrated by a Scree Plot (Fig. 3.6). The second principal component contributed 23% and together both principal components explained 81% towards total % variance within clade A (Table 3.9). The inclusion of the number of principal component is purely dependent on the criterion used. The criteria include: the scree plot criterion which looks for the "elbow" on the curve to determine the number of components to consider before it flattens; criterion based on proportion of variance explained which considers at least 75% explained by the principal components as well as the eigenvalue criterion which considers the principal components with an eigenvalue greater or equal to 1 (Boehmke and Greenwell 2019). The variables:

F_{max}, F_{peak} and S_{dur} had positive loadings on PC1 (Fig. 3.7). On the other hand, PC2 had a positive loading of S_{dur} (Fig. 3.7). There were significant differences between the genera within clade A using PC1 and PC2 (one-way ANOVA; PC 1: $F_{1.90} = 3.245$, $P < 0.05$, PC2: $F_{1.90} = 4.98$, $P < 0.01$), and *Post-hoc* Tukey's HSD test showed that within PC1, genus *Alaudala* differed significantly ($P < 0.05$) from *Galerida* and *Spizocorys* (Table 3.10). On the other hand, within PC2, genus *Galerida* differed significantly ($P < 0.05$) from *Melanocorypha* and *Spizocorys*; and *Lullula* differed significantly ($P < 0.05$) from *Eremophila* and *Alauda*, and ($P < 0.01$) from *Melanocorypha* and *Spizocorys* (Table 3.10). The song strophe from all three sampled genera within clade A differed significantly from one another (MANOVA: $F_{4,546} = 2.7312$, $P < 0.001$) and the greatest difference was between *Lullula* ($P < 0.01$) from *Melanocorypha* and *Spizocorys*.

The DFA's partition plot was able to classify 52% of the song strophes to the correct genus (Fig. 3.8), indicating that there were significant differences between all the genera sampled. The partition plot (Fig. 3.8) shows that the genera that were classified correctly were *Galerida, Calandrella, Alaudala, Melanocorypha, Spizocorys* and *Lullula.* Genus *Melanocorypha* (G) was grouped with *Eremophila* (C) and *Alauda* (D) in its cluster although some species in this genus were classified as *Alaudala* (F) and *Spizocorys* (H). Genus *Chersophilus* (E) was incorporated in *Calandrella.* Members of *Calandrella* (B) were classified as *Galerida* (A) and two species from *Galerida* were classified in the *Calandrella* cluster.

Clade *B* – the Mirafrid species

The univariate one-way ANOVA conducted using the genera located in clade B, yielded four variables (F_{max}, F_{min}, F_{peak} and S_{dur}) that differed significantly ($P < 0.05$) within clade B (Table 3.7). F_{min} returned the highest significant difference ($P < 0.01$) within clade B. *Post-hoc* Tukey's HSD test for the four variables (F_{max}, F_{min}, F_{peak} and S_{dur}) conducted in ANOVA test (Table 3.8). *Post-hoc* Tukey's HSD test on maximum frequency (F_{max}) showed that *MirafraN* (*MirafraN* are those species found out of Africa and are closely related) differed significantly at $P < 0.05$ from *MirafraS* (*MirafraS* are species that are found in Africa and they are closely related). On peak frequency (F_{peak}) *MirafraS* differed significantly at $P < 0.01$ and $P < 0.05$ from *MirafraN* and *Calendulauda* respectively. On Minimum frequency

(F_{min}), *MirafraN* differed significantly at $P < 0.05$, $P < 0.01$, $P < 0.01$ from *Calendulauda, Heteromirafra* and *MirafraS* respectively. Since there were more than two variables significantly different within the clade, only F_{band} and N_{ele} were excluded in the Principal Component Analysis.

Following an examination of a Scree Plot, PCA was extracted only one principal component with an eigenvalue of > 1.0 explaining 72% (Fig. 3.9). The second principal component contributed 15% and both components combined explained 87% of the total % variance within clade B (Table 3.9). F_{max}, F_{min}, F_{peak} and S_{dur} had positive loadings on PC1 (Fig. 3.10) while PC2 had a positive loading of S_{dur} (Fig. 3.10). There were significant differences between the genera within clade B using PC1 (one-way ANOVA; PC 1: $F_{1.90} = 8.766$, $P < 0.001$) and there were no significant differences for PC2. The *Post-hoc* Tukey's HSD test showed that within PC1, genus *MirafraN* differed significantly from *MirafraS* and *Heteromirafra* ($P < 0.001$ and $P < 0.05$ respectively) (Table 3.10). Genus *Calendulauda* did not differ significantly against *Heteromirafra,* both *Mirafra* (N and S groups).

Strophes from all three sampled genera within clade B differed significantly from one another (MANOVA: $F_{4,546} = 3.7845$, $P < 0.001$). The DFA was able to classify 68% of the song strophes to the correct genus within clade B (Fig. 3.11), indicating that there were significant differences among the genera sampled. The partition plot (Fig. 3.11) shows that only genus *Heteromirafra* (L) was incorrectly classified and joined *MirafraS* (J). The two groups (*MirafraS* and *MirafraN* (K)) were separated from each other and no species belonging to either group that was classified on the other group. *Calendulauda* (M) cluster had individuals from *MirafraS* and *MirafraN* and some of the species belonging to this genus were classified in both above-mentioned groups.

Clade *C* – the Ammomanid species

One-way ANOVA revealed that only two variables (N_{ele} and S_{dur}) differed significantly ($P < 0.001$) within clade C (Table 3.7). S_{dur} returned the highest significant difference ($P < 0.0001$) within clade C while N_{ele} differed significantly at $P < 0.001$. *Post-hoc* Tukey's HSD test for the two variables (N_{ele} and S_{dur}) conducted in the ANOVA test is presented in Table 3.8. To conduct PCA for clade C, Spearman rank correlation ($P < 0.05$) was conducted and four variables that correlated more than once (N_{ele}, S_{dur}, F_{peak} and F_{max}) were retained for

the analysis. Spearman rank correlation was used due to less variability within the clade using one-way ANOVA. From the above-mentioned set of correlated variables, N_{ele} and S_{dur} had the highest significance (Spearman rank correlation: $P < 0.001$), while the other two variables only passed the $P < 0.05$.

Following an examination of the Scree Plot (Fig. 3.12), two principal components with eigenvalue > 1.0 were extracted and these explained 84% of the total % variance in the data (Table 3.9). F_{max}, N_{ele}, F_{peak} and S_{dur} had positive loadings on PC1 (Fig. 3.13). On the other hand, PC2 had a negative loading of S_{dur} and positive loadings for F_{peak} and F_{max} (Fig. 3.13). There were significant differences between the genera within clade C using PC1 (one-way ANOVA; PC1: $F_{1.90} = 4.236$, $P < 0.05$) and PC2 had no significant differences. The *Post-hoc* Tukey's HSD test showed that within PC1, genus *Alaemon* differed significantly ($P < 0.05$) from *Certhilauda* (Table 3.10).

Song strophes from all seven sampled genera within clade C differed significantly from one another (MANOVA: $F_{4,546} = 3.5257$, $P < 0.01$) and the greatest difference was between *Alaemon* ($P < 0.05$) and *Certhilauda*. The DFA was able to classify 71% of the songs to the correct genus within Clade C (Fig. 3.14), indicating that there were significant differences among the genera sampled. What was observed in partition plot was that *Certhilauda*, *Eremopterix*, *Ammomanopsis* and *Alaemon* were classified as clusters. *Ammomanes* and *Ramphocorys* were classified in *Certhilauda* and *Chersomanes* was classified in *Eremopterix*. Only one individual from *Eremopterix* was classified in *Certhilauda* (Fig. 3.14).

3.3.4 Description of song strophes

The strophes with bolded names in Appendix 3.2 were used as references which means that they represent the exact or near description of the songs of the respective species as guided by the literature. In some instances, there exist intraspecific variation as visualised across multiple strophes of respective species.

Clade A

This clade consists of 10 genera of which nine genera were represented in this study. None of the species studied in this clade incorporates wing clapping.

Genus: *Galerida*

The genus *Galerida* was represented by six species (Appendix 3.2.1) namely, *G. cristata*, *G. theklae*, *G. malabarica, G. deva*, *G. macrorhyncha* and *G. magnirostris* which inhabit dry, stony areas with sparse shrubby vegetation (Alström 2019; de Juana *et al.* 2020). *Galerida magnirostris* and *G. theklae* are the only two that also inhabit grasslands. For each of the species, multiple strophes from different individuals were studied (Appendix 3.2.1a - f). Males in this genus generally render their song from the ground, in-flight or a low perch. Aurally and visually, the song strophes of *G. cristata, G. malabarica, G. theklae, G. deva,* and *G. macrorhyncha* are generally similar with *G. magnirostris* being different from the other species by having strophes that are made up of rolling notes with a distinguishable grouped element at the end of the strophes.

Members of *Galerida* have short strophes (≤4 s) with several elements and a distinctive character found in this genus is that the strophes end with a grouped element which is found in *G. cristata, G. theklae* and *G. magnirostris*. *Galerida theklae* and *G. magnirostris* have a warbling or bubbling grouped elements at the end of the strophes while it is trilling in *G. cristata*. Aurally, *Galerida* spp. strophes are predominantly tonal or musical (though with some trilling elements across the species in some individual strophes), with an ascending pitch in *G. magnirostris*, *G. cristata* and *G. malabarica* and a descending pitch in *G. theklae*, *G. deva* and *G. macrorhyncha.* Mimicry is known to occur in all the studied species except in *G. macrorhyncha* (Table 3.2).

Genus*: *Calandrella

The genus *Calandrella* was represented by four species (Appendix 3.2.2): *C. cinerea, C. brachydactyla*, *C. acutirostris* and *C. erlanger*. They all thrive in dry areas with sparse and low vegetation cover to open grassland with lots of bare ground (de Juana *et al.* 2020). Songs in this genus are often performed as in-flight displays and less often from the ground. The song strophes in this genus are predominantly musical with short strophes (≤4 s) which start with simple high-pitched elements and followed by a quick jumble of low-pitched elements (Table 3.2). Three species, *C. cinerea, C. brachydactyla* and *C. Erlanger*, have a descending pitch but *C. acutirostris* has an ascending pitch. None of the strophes of these

species end with grouped elements and they are known to imitate the songs of other bird species.

Genus*: *Eremophila

Two species represented the genus *Eremophila* (Appendix 3.2.3) namely, *E. alpestris* and *E. bilopha*. These species occupy a wide range of habitats: *E. bilopha* prefers open flat plains mainly on the edges of the true desert, while *E. alpestris* has managed to successfully colonise tundra and alpine habitats. Throughout their range they prefer mainly barren terrain with very short vegetation. The song is usually performed as an in-flight display, but in *E. bilopha* it is commonly performed from the ground (de Juana *et al.* 2020).

The song strophes of *E. alpestris* consist of a few simple, rippling trills followed by short chatter, less fluent than that of many other larks (de Juana *et al.* 2020), whereas the song of *C. bilopha* consists of a series of soft, usually short, rather monotonous twittering and warbling phrases with short whistles (Table 3.2). From the sampled songs, the strophes are short (≤4 s) consisting of about 3 to 7 introductory elements, followed by a jumble of warbling grouped elements at the end. The grouped elements are present in *C. alpestris* but absent in *C. bilopha*. Although the strophe has trilling and whistling features, it is predominantly musical aurally and has an ascending pitch. There is no existence of published record of mimicry in literature.

Genus*: *Alauda

Only one species represented *Alauda* (Appendix 3.2.4), namely *A. leucoptera* which occurs in open, temperate grass or wormwood steppe and in cultivated areas (Donald 2004, Alström 2019). One good quality strophe of *A. leucoptera* was analysed and this cannot be interpreted as sound representation for the song of this species. The song is uttered from the ground or low perch and during an in-flight display and it is made up of a mixture of twittering and trilled elements. The analysed song strophe consists of eight elements, the first six elements that are clear whistles and two harsh elements at the end characterised by trills (Table 3.2). The strophe is short (≤4 s), predominantly musical with a descending pitch. The strophe is characterised by the absence of warbling grouped element ending and there is no existence of published record of mimicry in literature.

Genus: *Chersophilus*

Chersophilus duponti (Appendix 3.2.5) occurs in open plains with shrub-steppe or feather grass. The song display occurs mostly at dawn and sunset from the ground or during an in-flight display. According to de Juana and Suárez (2020), the song comprises a series of twittering with buzzing elements often lasting up to 30 minutes or more. Two song strophes with two to three repeated elements that rise in pitch lasting approximately 2.73 to 6.22 s were analysed. The strophe has an intermediate length with the duration falling in the range of 4.1 – 8 s consisting of an ascending pitch and being predominantly musical (Table 3.2). The strophe does not end with a grouped element and there is no published record of mimicry in literature.

***Genus:* Alaudala**

Genus *Alaudala* (Appendix 3.2.6) comprises four sampled species, namely *Alaudala rufescens*, *A. somalica*, *A. raytal* and *A. cheleensis.* These species prefer dry open grasslands except *A. raytal* which inhabit in dry, sandy riverbanks and flood plains of lakes. The general song strophe is usually performed in a high in-flight display, but also from the ground or a low perch (Table 3.2). The strophe of *A. rufescens* is a varied, continuous melody mixed with rattles, chirrs, trills and whistles with squeaking sounds, buzzy and grating elements, *A. somalica* has a protracted series of trills and whistles in its strophe, whereas the strophe of *A. raytal* consists of a few single elements interspersed with fairly long (often 10 s or more) pauses, resembling that of *A. rufescens* but slightly less varied. The strophe of *Al. cheleensis* is similar to that of *A. rufescens*.

The duration of the strophe of *A. raytal* is 4.88 s with an ending trill of spaced elements. *Alaudala cheleensis* and *A. rufescens* have a predominantly musical, relatively long song strophe (> 8 s) with an ascending pitch while *A. somalica* and *A. raytal* have intermediate (4.1 – 8 s) strophes that are predominantly musical (except *A. somalica* which has a harsh or screeching strophe) with a descending pitch (Table 3.2). All the analysed species except *A. somalica* are characterised by ending their strophes with warbling grouped elements. One species, A. rufescens, is known to perform heterospecific mimicry (de Juana *et al.* 2020).

Genus: Melanocorypha

The genus *Melanocorypha* is represented by four species (Appendix 3.2.7), *Melanocorypha calandra*, *M. bimaculata*, *M. maxima* and *M. yeltoniensis*. These species occur in open habitats, generally with stones and less grassy terrain. Song performance is usually in-flight; from the ground or a low perch such as bush. The strophe of *M. calandra* is as described in the literature and gives a drawn-out medley of rolling trills mixed with harsh sounds (de Juana *et al.* 2020). *Melanocorypha bimaculata's* strophe is a prolonged, fast twittering like *M. calandra* but simpler, harsher and more grating (Alström 2019) (Table 3.2). The strophe of *M. maxima* is rich and varied, rather slow pacing (Alström 2019) whereas *M. yeltoniensis* has a rapidly twittering and chirping strophe with separated softer, more mournful elements, similar to that of *M. calandra* (Alström 2019). *Melanocorypha calandra, M. bimaculata*, *M. maxima* and *M. yeltoniensis* all have a varied length of song strophes that is either intermediate (4.1 – 8 s) and ascending in pitch, short (≤ 4 s) and ascending in pitch, long and descending (> 8 s) and short (≤ 4 s) and descending in pitch respectively. The strophes are predominantly musical ending with grouped elements (trilling in *M. calandra* and *M. maxima*, warbling in *M. yeltoniensis*) except in *M. bimaculata* where it is harsh and lack a grouped element ending. All species except *M. yeltoniensi*s are known to mimic other species.

Genus: Spizocorys

Spizocorys was represented by four species (Appendix 3.2.8), namely, *S. conirostris*, *S. fringillaris*, *S. starki*, *S. sclateri.* These species are found in habitats ranging from arid, semi-arid grass plains to moist, sub-montane grasslands and savanna. The song displays may be performed in-flight or from the ground while foraging. The song strophe across this genus is characterised by short, screeching elements that sound more like calls than songs (Ryan 2019). Only one individual strophe for all spp. in this genus was analysed due to scarcity, as these birds rarely display their songs. The song of *S. conirostris* largely consists of short, sweet, whistled elements, often repeating each element 3–4 times, *S. fringillaris song* strophe comprises a dry series of rapid 9 to 10 elements, *S. starki* strophe is a rambling series of rather unmelodic chirps and trills, with an occasional sweeter whistle while *S. sclateri* strophe is a soft "prrp prrp" or "prrp prrp treep".

All the species have short strophes (≤4 s) with stable pitch and are predominantly musical except *S. sclateri* and *S. starki* where they are screeching. All the species lack grouped element ending and there is no published record of mimicry in literature.

Genus: Lullula

Lullula is a monotypic genus containing *L. arborea* (Appendix 3.2.9) which inhabit a variety of open and semi-open habitats. The song is uttered in-flight, or from the ground or low perch, and sometimes at night. The strophe is relatively short (≤4 s), predominantly musical and with clear, melodious series of elements that turn into faster elements. The strophe lacks a grouped element ending and there is no published record of mimicry in literature.

Clade B

This is a clade which is comprised of three genera of which all of them were studied in this chapter:

Genus: Mirafra

The genus was represented by 17 species, namely, *Mirafra africana*, *M. hypermetra*, *M. angolensis*, *M. gilletti*, *M. pulpa*, *M. cheniana*, *M. passerina*, *M. apiata*, *M. fasciolata*, *M. rufocinnamomea*, *M. javanica*, *M. cantillans*, *M. microptera*, *M. affinis*, *M. assamica*, *M. erythroptera* and *M. erythrocephala*. These species are found in a wide range of habitats, ranging from open habitats such as grasslands to fairly densely vegetated habitats.

Visually, all the studied species in genus *Mirafra* have song strophes that have predominantly whistling features with the exception of *M. angolensis, M. gilletti, M. passerina* and *M. assamica* that have harsh or screeching strophes (Fig. 3.3.1, Table 3.2). The strophes are either short (≤4 s) for all or intermediate (4.1 – 8 s) with an ascending pitch in *M. angolensis*, *M. erythrocephala*, *M. cantillans, M. affinis* and *M. erythroptera.* Generally, the strophes were found to have an ascending pitch but either stable in *M. rufocinnamomea* and *M. cheniana* or descending in *M. africana* and *M. gilletti*. All the strophes lack the warbling grouped element at the end except *M. javanica* and *M. microptera.* Wing clappings are present in the strophes of *M. africana, M. rufocinnamomea, M. fasciolata* and *M. apiata* even though they are not incorporated in the actual song

strophes in *M. africana* and *M. rufocinnamomea* unlike in *M. fasciolata* and *M. apiata*. From the studied *Mirafra* species, eight of them (*M. africana*, *M. cheniana*, *M. hypermetra*, *M. rufocinnamomea*, *M. fasciolata, M. apiata, M. javanica* and *M.* assamica) are known to mimic sounds of other bird species (de Juana *et al.* 2020).

The song strophe of *M. africana* is short and simple often instantly preceded by wing clappings and repeated several times. Contrary to the two elements mentioned in literature, *M hypermetra* renders six elements during short song aerial display (de Juana *et al.* 2020). As reported in de Juana *et al.* (2020) the song is like that of *M. africana* but long and loud. The song strophe of *M. angolensis* (Appendix 3.3.1c) consists of a series of typical, buzzing trills that vary in pitch, given in aerial display and sometimes from the ground (de Juana *et al.* 2020). *Mirafra gilletti* is represented by only one strophe and cannot be interpreted as sound representation of the song of this species. This species is little known and renders a long song in the aerial display (de Juana *et al.* 2020). Aurally, this strophe sounds like a *M. hypermetra* song strophe. *Mirafra pulpa* renders a stereotyped whistled song from an elevated perch and it gets repeated (de Juana *et al.* 2020). *Mirafra cheniana*'s song strophe is usually delivered in the aerial display but also from a low perch, and comprised of long elements that alternate in pitch. *Mirafra passerina* song may be rapidly delivered from elevated perch or in short display-flight incessantly singing a croaking, gurgling, distinctive four-note "for-syrup-is-sweet" song every 3-5 seconds for hours on end (de Juana *et al.* 2020). Five strophes from different individual birds of *M. apiata* were analysed. The *M. apiata* song is somehow replaced by the aerial wing clapping display, followed by a simple, ascending whistle. Five individual strophes of *M. fasciolata* were analysed. The song strophe of *M. fasciolata* is like that of *M. apiata* as it progresses with the wing clapping display but longer, followed by a somewhat ascending whistle contrary to a descending whistle as described in the literature (Ryan 2019). *Mirafra rufocinnamomea* strophe is a thin, rather faint whistle consisting of at least three elements.

Mirafra javanica's song strophe is delivered from a perch or in flight, consisting of introductory elements sparsely separated followed by some compressed elements forming a slow trill. The strophe of *Mi. cantillans*, has a series of chirps, whistles and buzzes like that of *M. javanica* as both species have double-element repetition before switching to another repetition. *Mirafra microptera*'s song strophe is as described in de Juana *et al.*

(2020) and is said to have a series of high-pitched whistles but the two introductory elements observed in this study (ID no. XC80381) were not described.

Mirafra affinis has an unusually long song rendered monotonously. The song is as described in de Juana *et al.* (2020) and consists of a dry and metallic rattle "zizizizezezezezezezezezezezezezezeze". *Mirafra assamica* song is as described in de Juana (2020) with a repetition of a thin, harsh, squeaky, disyllabic elements. The length of each element of *M. assamica* ranges from 0.40 to 0.58 s. *Mirafra erythroptera* song strophes consist of clear whistles interchanging in pitch and length while *M. erythrocephala* song is as described by de Juana *et al.* (2020), consisting of quick, thin, clear, mostly drawn-out notes, and almost sounds like a "siren".

Genus: *Heteromirafra*

This genus is represented by one species only in this study, namely *Heteromirafra ruddi* (Appendix 3.3.2) which occurs in short upland grassland, usually near damp depressions. The song is usually given in the aerial display or from the ground. The strophe is short (≤4 s), predominantly musical and has a relatively stable pitch (Table 3.2). The strophe does not end with a warbling grouped element, lacks wing clapping and there is no published record of mimicry in literature.

Ge*nus: Calendulauda*

The genus *Calendulauda* (Appendix 3.3.3) is represented by seven species: *Calendulauda barlowi*, *C. erythrochlamys*, *C. albescens*, *C. burra*, *C. africanoides*, *C. alopex* and *C. sabota.* A majority of species in this genus prefer sandy habitats with the exception of *C. sabota* which prefers savanna and open woodland.

The song strophes of all the studied species are predominantly musical, short (≤4 s) and generally have an ascending pitch except in *C. burra, C. africanoides* and *C. sabota* which have a descending pitch. *Calendulauda barlowi, C. erythrochlamys, C. albescens* and *C. burra* song strophes end in a trilling grouped element ending while no grouped element ending is observed in *C. africanoides, C. alopex* and *C. sabota.* Wing clappings are absent in all the species mimicry has been reported in *C. africanoides, C. alopex* and *C. sabota.*

Four strophes of *Calendulauda barlowi* have 6 to 9 ‘staccato’ clicking elements followed by a buzzing trill (Table 3.2). As described in de Juana *et al.* (2020), *C. erythrochlamys* sing during aerial display or from the ground or a bush. The strophe is a long series of 10–13 simple elements followed by a brief whistle and then a rapid trill. Alström and de Juana *et al.* (2020) maintain that *C. albescens* renders the song during aerial display or from the ground or a bush. The song strophe is a stereotyped phrase of 2–5 staccato elements followed rather by a buzzy trill. Concerning *C. burra,* the song strophe is a series of elements. This species’ song is superficially like *C. albescens* but deeper and slower, and with a significant difference in the leading elements and how they are structured. The terminal trill is somewhat complex, comprised of three repeated multi-component elements.

Calendulauda africanoides sings from elevated perches or during an aerial display. Typically, its song is rapid and has an accelerating series of high-pitched, scratchy elements that are monotonously repeated. *Calendulauda alopex*’s song strophe is hurried like that of *C. africanoides*, usually given from the top of a small tree or other elevated perch, or in flight. Five song strophes of *C. sabota* strophes were analysed. The strophe of *C. sabota* is long and rambling, usually from an elevated perch or in display-flight.

Clade C

Clade C consists of eight genera of which seven of them were studied in this chapter:

Genus*: Certhilauda*

The genus *Certhilauda* (Appendix 3.4.1) is represented by six species, *Certhilauda brevirostris*, *C. curvirostris*, *C. semitorquata*, *C. benguelensis*, *C. subcoronata* and *C. chuana*. Members of this genus prefer habitats ranging from coastal dunes and fynbos to open grassland and savanna. Generally, the song in the genus is a simple whistle preceded by an introductory element. All the species analysed (*C. brevirostris*, *C. curvirostris*, *C. semitorquata*, *C. benguelensis*, *C. subcoronata*) render predominantly musical and short song strophes (≤4 s), all with descending pitch except for *C. chuana* (Table 3.2). None of the *Certhilauda* spp. include wing clapping in their song strophes and there is no record of mimicry documented in the literature (Ryan 2019).

The species *C. brevirostris* was found to have a song strophe that is a loud two-element whistle. *Certhilauda curvirostris'* typical song is given in flight display or from a low perch or from the ground, with the introductory element being much shorter, softer and less far-carrying than the second element. The introductory element is shorter and less pronounced than in song of *C. brevirostris*. There is however variation in that sometimes the song is rendered with no introductory element. *Certhilauda semitorquata* song is given throughout the year, from a perch such as a rock or in an aerial display. This species gives a song strophe which is simple and has a descending pitch and it is like that of *C. subcoronata* both aurally and visually. *Certhilauda benguelensis* song strophe is a whistle which superficially resembles that of *C. curvirostris*, *C. brevirostris* and *C. subcoronata* but has a soft introductory element. *Certhilauda subcoronata* renders a two-element song strophe which is an ascending whistle preceded by a soft introductory element. The typical song strophe of *C. chuana* is a simple whistle preceded by a soft introductory element. There is variation in that one or two or three whistle elements can succeed in the introductory element (Mashigo *et al.* 2018, unpbl. Mini-dissertation). Sometimes the introductory element is omitted.

Genus: *Eremopterix*

The genus *Eremopterix* (Appendix 3.4.2) is represented by five species, *Eremopterix nigriceps*, *E. griseus*, *E. signatus*, *E. leucotis* and *E. verticalis.* Members of this genus prefer habitats ranging from semi-arid and arid plains with grassland, savanna, and clearings in open woodland (Ryan 2019). They mainly give short song strophes (≤4 s) (*E. nigriceps*, *E. griseus*, *E. signatus, E. leucotis)* except *E. verticalis* with an intermediate song strophe (4.1 – 8 s) which is predominantly harsh or screeching and descending in pitch (Table 3.2). *Eremopterix nigriceps* and *E. leucotis* have predominantly musical strophes that are respectively descending and ascending in pitch while *E. griseus* and *E. signatus* strophes are predominantly screeching and respectively ascending and descending in pitch. All the species have strophes that lack the grouped element at the end as well as wing clappings and there is known record of heterospecific mimicry documented in the *E. leucotis* (Engelbrecht and Dikgale 2017). Despite the variability observed across *Eremopterix* spp., *E. signatus* has a short twittering song strophe consisting of a series of rising, mournful piping elements*. Eremopterix leucotis* song strophe is monotonous though giving sweet,

high-pitched whistles. The *E. verticalis* song strophe is mostly given in aerial display, a series of simple elements.

Genus: Ammomanes

Genus *Ammomanes* (Appendix 3.4.3) is represented by two species, *Ammomanes cintura* and *A. deserti.* The two spp. from this genus are said to prefer semi-desert to desert regions with less than 100 mm annual rainfall, also semi-deserts, in flat or gently undulating terrain, stony or sandy soils, and very sparse or almost no vegetation cover. These species prefer gravel plains with mixed sandy areas, with grasses, and small depressions in the terrain; common in semi-arid savanna. The *A. cintura* song strophe has short whistles, the first element is lower in pitch and often audible only at a short distance. The last element is high, pure and squeaky. This species renders a predominantly musical, short strophe (≤4 s) which ascends in pitch. The strophe does not end in a grouped element, lack the presence of wing clapping and there is no published record of mimicry in literature. *Ammomanes deserti* renders its song strophe in flight, or from the ground. The strophe is predominantly musical, short (≤4 s) and ascends in pitch. The strophe does not possess a grouped element at the end, it lacks the wing clappings and there is no published record of mimicry in literature.

Genus: *Ramphocoris*

Ramphocoris is a monotypic genus consisting of *R. clotbey* (Appendix 3.4.4) which inhabit borders of deserts, including true desert, with a preference for stony or compact soils. The song strophe is rendered from the ground or in flight, which can be described as a soft and rather quiet medley of tinkling elements (de Juana *et al.* 2020). This species has a strophe which is predominantly musical, short (≤4 s) and with a descending pitch (Table 3.2). The strophe does not end in a grouped element, lacks the presence of wing clapping and there is no published record of mimicry in literature.

Genus*: Ammomanopsis*

This is a monotypic genus *Ammomanopsis* (Appendix 3.4.5), which contains *A. grayi.* This species inhabits gravel plains, clayey soils, and salt flats with little or no vegetation. Other than scattered grass clumps or patches of succulents, this species avoids drifting sands

(Ryan 2019). *Ammomanopsis grayi* renders the song that is given from ground or during in-flight displays, mostly before dawn or after dusk. This species has a predominantly musical, short (≤4 s) strophe with an ascending pitch (Table 3.2). The strophe does not have a grouped element at the end, lack wing clapping and there is no published record of mimicry in literature. *Ammomanopsis grayi* gives a high-pitched strophe with a series of short elements and ascending whistles, and various short whistles, typically ascending in pitch.

Genus*: Chersomanes*

The genus *Chersomanes* (Appendix 3.4.6) has two species and in this chapter, it is represented by *C. albofasciata* which inhabits a range of open habitats, from montane grassland to arid Karoo shrublands and semi-desert and desert plains (Ryan 2019. The song strophe is predominantly musical and has a short strophe with a descending pitch. The strophe does not have a grouped element at the end, it lacks wing clappings and there is no published record of mimicry in literature.

Genus*: Alaemon*

This genus *Alaemon* (Appendix 3.4.7) consists of two species and here it is represented by *A. alaudipes* which inhabit deserts or semi-deserts, in open plains or rolling terrain, with sandy soils and sparse vegetation cover; also, in areas with a mixture of gravel and sandy soils (Ryan 2019). De Juana *et al.* (2020) maintains that the song strophe is uttered during in-flight display, forming a series of uniform, melodious and piping sounds. It starts slowly and accelerates as it climbs, then a short trill at the peak of the ascent, followed by a further series of whistles ascending in speed and tone. The strophe of *Ala. alaudipes* is predominantly musical, it is long (>8 s) and has an ascending pitch (Table 3.2). There is no grouped-element ending in strophe, no wing clappings in the strophe and there is no published record of mimicry in literature.

3.4 Discussion and Conclusions

Are the major clades distinct?

It is clear from the findings that among the three clades (A - the Alaudid, B - Mirafrid and C – the Ammomanid) as identified in Alström *et al.* (2013), the distinction was found between the song strophes of clade C and A species. The song strophes of clade A and B species largely overlapped and were insignificantly different. The Alaudid species in clade A generally give strophes that are defined by high maximum frequency as well as high peak frequency and they have broad bandwidth frequency. The species in clade B have a similar trend with those species belonging to clade A, hence they are not statistically significantly different from each other. The majority of species from clade A and B are found on the positive of PC1 in the biplot analysis and the direction of influence is from the three variables (high maximum frequency, high peak frequency, broad bandwidth frequency) that were found to differentiate the three clades. These statistical findings are in support of the findings in Alström *et al.* (2013) and this study. Phylogenetically, clade A and B shared a sister relationship while clade C was placed basally (Fig. 1.2). Clade C, on the other hand, comprises species that have song strophes that are defined by low maximum frequency, lower peak frequency and narrow bandwidth frequency and this clade differed significantly from clade A with the majority of species in this clade being on the negative of PC1.

Despite that not all of the species could be correctly classified in their respective clades, the largest number was for the species that were correctly classified in the respective clades mostly in clade A and C. In addition, the distinction among the clades was also observed in either the presence or the absence of wing clappings in the song strophes that is, either being detached from the song strophe or incorporated or attached to the song strophe. Clade B is the only one which is marked by the presence of wing clappings, in particular, genus *Mirafra*. Clade A and C song strophes lack wing clappings.

Does the grouping of taxa compare to the phylogenetic grouping?

This distinctiveness of the three major clades was further supported by analysis of grouping of taxa within each clade independently. The non-African *Mirafra* species (*MirafraN)* in clade B were, however, separated from clade C taxa, *Certhilauda* and *Ammomanes.*

Alaemon (clade C) emerged distinct from *Eremophila* and *Spizocorys* (clade A) and both non-African *Mirafra* species (*MirafraN)* and African *Mirafra* species (*MirafraS)* (clade B). Despite some level of overlap between the clades, the statistical results on quantitative parameters support the distinctiveness at least between clade C and A.

As far as the investigation on the uncertain or unresolved phylogenetic placement of taxa in Alström *et al.* (2013) is concerned,

i) the unexpected sister relationships between the two monotypic genera *Chersophilus* and *Eremalauda* and between this clade (the two species) and *Alaudala* complex reported in Alström *et al.* (2013) is equally surprising as *Chersophilus* is separated from all *Alaudala* species in all the analysis. Genus *Eremalauda* was not part of the analysis and its statistical placement is unknown. The *Calandrella* complex and *Eremophila* sister relationship that was unexpected in Alström *et al.* (2013) is equally surprising in this study as *Eremophila alpestris* is separated from the *Calandrella* complex. On the other hand, *E. alpestris* unlike all the other species in genus *Calandrella* and *Eremophila* is the only one that has song strophes that end with a grouped element.

(ii) The inconclusive results concerning the relationships between the three species of *Alauda* in Alström *et al.* (2013) cannot be commented on as only one species, *Alauda leucoptera* was included in this study.

Regarding the incongruent topologies in clade A as outlined in Alström *et al.* (2013), this study found that in clade A, genus *Alaudala* was significantly different from *Galerida* and *Spizocorys*, while *Alaudala* has a distant relationship from the latter in both topologies. Genus *Galerida* also differed significantly from *Melanocorypha*. In this study, *Lullula* differed significantly from *Eremophila*, *Alauda*, *Melanocorypha* and *Spizocorys* and in the two topologies, Cyt*b* represent these separations better than ODC topology although there is a close relationship between *Lullula* and *Spizocorys* in Cyt*b* topology. What is unique is the presence of grouped element (warbling or trilling) in the strophes of some of *Galerida* and *Spizocorys* species

(iii) In Alström *et al.* (2013) genus *Spizocorys* incorporated a species that was previously in a monotypic genus *Pseudalaemon* that is, *Pseudalaemon fremantlii,* and half of the species from *Spizocorys* were represented by 16S and Cytb sequences.

Unfortunately, in this study, the songs of *Spizocorys fremantlii* was not available and therefore no comment on its placement in this genus can be made. However, the findings in this study indicates a close association between *S. conirostris, S. sclateri* and *S. starki* while *S. fringillaris* is pulled away from the other species in this genus.

(iv) In clade B, the relationships within clade B2a apart from the sister connection between *Calendulauda barlowi* and *C. erythrochlamys* are viably unresolved. In this study, these prior species are not significantly different from *C. albescens* at clade level and the unresolved *C. burra* is significantly different from *MirafraS* (*Mirafra apiata, Mi. cheniana*). What came out of the structural analysis of the songs in genus *Calendulauda* is that the southern African species (*C*. *albescens*, *C*. *barlowi*, *C*. *erythrochlamys*) commonly possess song strophes that end with a trilling grouped element and are all not known to mimic other species. The absence of trilling grouped element in the southern African *Calendulauda* taxa is found in *C*. *burra* which also does not mimic other species, while *C*. *africanoides* and *C. sabota* also lack the trilling grouped element but mimic other species. On the other hand, the northern African *Calendulauda* species (*C. alopex*) has strophes that lack grouped element at the end of the song strophes and no record of mimicry has been published in literature.

In clade B1a, the relationship in Alström *et al.* (2013) of the five Asian taxa (*Mirafra affinis*, *M*. *erythroptera*, *M*. *erythrocephala*, *M*. *assamica* and *M*. *microptera*) was unresolved wherein 16S sequences were unavailable. Vocally, these species presented a challenge in this study in terms of selecting what is defined as a song strophe due to the continuous nature of the songs. Unlike in the other Alaudidae species, there are no obvious strophes. Therefore, characterisation of these species based on songs was not explored as done in the other species. The spectrograms were based on the subjective selection of what could be considered a strophe for analysis. Consequently, the outcomes of the analysis of these species may not be counted. From this study, *M. erythrocephala, M. erythroptera, M. affinis* and *M. microptera* were completely separated from *M. assamica.* These species were also scattered from each other, the biplot places them on the positive of PC1 as variables, indicating that they possess the highest frequencies than *M. assamica*. (v) In Alström *et al.* (2013)'s C1a, a clade containing five species of *Eremopterix* is well supported, although the relationships among these are effectively uncertain. In our findings,

Eremopterix leucotis and *E*. *griseus* had song strophes with lower peak frequencies and lower maximum frequencies. *Eremopterix nigriceps* had long song strophes, while *E*. *signatus* and *E. verticalis* are separated by PC2 in the clade level analysis. This implies that the song strophe of *E*. *verticalis* has longer and more elements, while the song strophe of *E. signatus* has a higher maximum frequency. Therefore, the results in this study place *E*. *leucotis* closer to *E*. *griseus*, while *E*. *signatus* and *E*. *verticalis* are more closely related than to *E*. *nigriceps*.

Areas of the findings contradicting what is in literature

In clade B, *Mirafra microptera* song strophe is described in the literature as lacking the two introductory elements that were observed in the current study. *Certhilauda chuana* is another species of which in literature is described as having a single ascending whistle contrary to the findings in this and Mashigo study (2018 – unpublished mini-dissertation). This species has a typical territorial call which consists of either one, two or three whistle elements preceded by an introductory element which sometimes gets omitted. Another area of contradiction was observed in the song strophe of *M. fasciolata* which is noted to have an ascending whistle as opposed to a descending whistle as reported in de Juana *et al.* (2020). In literature, *Alaudala raytal* is reported to have a strophe duration of < 4 s, but in this study, the song strophe is > 4 s. This may be due to the finding that the first introductory element may have been omitted in literature. *Spizocorys fringillaris* is said to give a dry “tchiree” while this was found to render 9 to 10 elements than a single element.

In conclusion, the song strophes of the Ammomanid species (clade C) were most different from those of clade A and B species. This could be attributed to the phylogenetic relationship shared by the three clades. Clade C is the most basal clade phylogenetically. As per the record in literature Clade A songs incorporate mimicries (excluded in this study) except for few cases in genera *Spizocorys*, *Eremophila*, *Chersophilus* and *Lullula*. In clade B, cases of mimicry were reported in *Mirafra* and *Calendulauda* except for *Heteromirafra*. On the other hand, only one species in clade C, *Eremopterix leucotis* was found to mimic as observed in this study. Therefore, mimicry seems to be quite prevalent in several species of larks.

Predominantly, the lark family consists of songs that are musical aurally. There are few cases, however, where the songs of larks include harsh or screeching components to the ear and visually. These cases were recorded specifically for few species as follows: in clade A, *Alaudala somalica*, *Melanocorypha bimaculata*, *Spizocorys sclateri* and *S. starki*; clade B: *Mirafra angolensis*, *Mirafra gilletti*, *Mirafra passerina* and *Mirafra assamica*; clade C: *Eremopterix griseus*, *Eremopterix signatus* and *Eremopterix verticalis*. Furthermore, 52% of songs in the lark generally ascend in pitch, 38% descend while only 10% of songs in larks have a stable pitch. Genus *Spizocorys* (clade A), *Mirafra* and *Heteromirafra* both in clade B, are the only taxa with stable songs, while clade C has no record of stable songs.

Genus *Calendulauda* from clade B resemble most of the song strophes in clade A in the sense that most of the song strophes in this clade have harmonics, the broad frequencies concerning genus *Mirafra*, also in clade B, which is represented by song strophes that are simple and whistled, resembling those of clade C. Another key aspect is the presence of grouped-elements found at the end of the song strophes. This is evident in most species in clade A and B, while clade C has no record of grouped-elements that end the strophes.

Overall, the strophe duration of songs of larks cannot be used to differentiate the clades. The lark family is characterised by short songs except for *Alaudala cheleensis, Al. rufescens, Melanocorypha maxima* (clade A) and *Alaemon alaudipes* (clade C) characterised by long song strophes. Few cases of intermediate song strophes were recorded in clade A: *Chersophilus duponti, Alaudala somalica, Alaudala raytal* and *Melanocorypha calandra*; clade B: *Mirafra angolensis*, *Mirafra erythrocephala*, *Mirafra affinis*, *Mirafra cantillans* and *Mirafra erythroptera* and clade C: *Eremopterix verticalis.* In this instance, clade A and B, again resemble each other compared to clade C.

It can be concluded that by using vocal data, species of larks could be characterised at clade level and to a certain extent at genus and species level. The distinctiveness of clade C from clade A has been demonstrated. Clade A and B overlaps largely and this probably could coincide with the phylogenetic affinity between species belonging to the two clades. Having excluded songs with mimicked components, this study, however, shows some potential in using vocalisations comparatively with the existing phylogenetic

outcomes in systematics. Based on the findings in this study, it is recommended that recordings of songs of the balance of species which were not studied should be included in the future and multiple songs from multiple individual birds per species should be analysed. Some species were not analysed in this study due to poor quality of song recordings. Therefore, more song recordings should be made and they should be made available in sound archives such as Xeno-canto, Cornell University's Macaulay Library of Natural Sounds (MLNS) and Avian Vocalization Centre (AvoCet) so that research on vocalisations can be possible.

TABLE 3.1. LIST OF SONG RECORDINGS FROM WHICH SONG STROPHES WERE SELECTED. MLNS = CORNELL UNIVERSITY'S MACAULAY LIBRARY OF NATURAL SOUNDS, AVOCET = AVIAN VOCALIZATION CENTRE, PA = PER ALSTRÖM, DA = DESMOND ALLEN, XC = XENO-CANTO. BOLDED NUMBERS INDICATE SONG RECORDINGS FROM WHICH REFERENCE SONG STROPHES WERE EXTRACTED.

Clade	Species Name	Common Name	Source/Recorder	Recording No.
Clade A -	*Alauda leucoptera*	White-winged Lark	Xeno-canto	**XC236707**
Alaudid	*Alaudala raytal*	Sand Lark	Per Alström	**PA_9104717**
	Alaudala somalica	Somali Short-toed Lark	Xeno-canto	**XC300030,** XC300031, XC300033
	Galerida magnirostris	Large-billed Lark	Xeno-canto	XC313733, XC280313,
			Per Alström	**PA_1002**, PA_1038
	Galerida cristata	Crested Lark	MLNS	**ML86284**
			Xeno-canto	XC361968, XC91636
			Per Alström	PA_91_02_303
	Galerida theklae	Theklae Lark	Xeno-canto	**XC267275**, XC300306, XC370396
	Galerida malabarica	Malabar Lark	Xeno-canto	**XC44811**
			Per Alström	PA_93_01_48-51
	Galerida deva	Sykes's Lark	Xeno-canto	**XC369214**, XC369215, XC369216, XC369217
	Galerida macrorhyncha	Maghreb Lark	Xeno-canto	**XC91636,** XC317662
	Spizocorys conirostris	Pink-billed Lark	Per Alström	**PA_1008**
	Spizocorys fringillaris	Botha's Lark	MLNS	**ML80706**
	Spizocorys starki	Stark's Lark	MLNS	**ML61156**
	Spizocorys sclateri	Sclater's Lark	MLNS	**ML61157**
	Eremophila alpestris	Horned Lark	Xeno-canto	XC293914
			MLNS	**ML73842**, ML118662, ML516603, ML50257
	Eremophila bilopha	Temminck's Lark	Xeno-canto	**XC175385**
	Calandrella cinerea	Red capped Lark	Xeno-canto	**XC279914**, XC279923, XC279922

			Per Alström	PA_2-1092
	Calandrella brachydactyla	Greater Short-toed Lark	Xeno-canto	**XC295988**, XC164215,
	Calandrella acutirostris	Hume's Short-toed Lark	Xeno-canto	**XC176708**
	Calandrella erlangeri	Erlanger's Lark	Xeno-canto	**XC300037**
	Melanocorypha maxima	Tibetan Lark	Xeno-canto	**XC110990,** XC110991
	Melanocorypha calandra	Calandra Lark	Xeno-canto	**XC268744**, XC243479
	Melanocorypha bimaculata	Bimaculated Lark	Xeno-canto	**XC257185**
	Melanocorypha yeltoniensis	Black Lark	Xeno-canto	**XC108873**, XC154499, XC145076
	Chersophilus duponti	Dupont's Lark	Xeno-canto	**XC140665,** XC315238
	Alaudala cheleensis	Asian Short-toed Lark	Xeno-canto	**XC3162713**
	Alaudala rufescens	Lesser Short-toed Lark	Xeno-canto	**XC175825**
	Lullula arborea	Wood Lark	Xeno-canto	**XC374316,** XC373126, XC374310, XC311724
Clade B - Mirafrid	*Mirafra apiata*	Cape Clapper Lark	Xeno-canto	**XC288867**, XC292640, XC292650, XC304509
			Per Alström	PA_1067
	Mirafra fasciolata	Eastern Clapper Lark	Xeno-canto	**XC280466**, XC292634
			Per Alström	PA_2017-1114, PA_2017-1117, PA_2017-1125
	Mirafra rufocinnamomea	Flappet Lark	Xeno-canto	**XC270466**, XC41316, XC266874
	Mirafra cheniana	Melodious Lark	Per Alström	**PA_1005**
			Dawie de Swart, GD Engelbrecht, A Nthangeni	DS20160923
			MLNS	ML72592
	Mirafra passerina	Monotonous Lark	Xeno-canto	**XC280457**, XC204673
				ML61171, ML61155, ML61146
	Mirafra angolensis	Angolan Lark	MLNS	**ML101195,** ML101192, ML101193

Mirafra hypermetra	Red winged Lark	Xeno-canto	**XC57941,** XC178954
Mirafra africana	Rufous-naped Lark	Xeno-canto	**XC268628,** XC280455, XC307246
		Per Alström	PA_1001, PA_1017
Mirafra gilletti	Gillett's Lark	MLNS	**ML100216**
Mirafra pulpa	Friedmann's Lark	MLNS	**ML8044**
Mirafra javanica	Horsfield's Bush Lark	MLNS	ML128310, ML128322
		Desmond Allen	**DA07_2_16**
		Per Alström	PA_87-679-720
		Xeno-canto	XC167898
Mirafra cantillans	Singing Bush Lark	Per Alström	**PA_98-01-40**, PA_98-01-39, PA_98-01-38
		Xeno-canto	XC267260, XC114273
Mirafra microptera	Burmese Bush Lark	Xeno-canto	**XC80381**, XC80380
Mirafra affinis	Jerdon's Bush Lark	Per Alström	**PA_93-01-18-21**, PA_93-01-23-28, PA_93-01-30, PA_93-01-23
		Xeno-canto	XC190819
Mirafra assamica	Bengal Bush Lark	Per Alström	**PA_94-01-60-61**, PA_94-02-14, PA_94-02-02, PA_94-02-22-28
Mirafra erythroptera	Indian Bush Lark	Per Alström	**PA_97-04-40**, PA_97_04_39, PA_91_01_006
Mirafra erythrocephala	Indochinese Bush Lark	Xeno-canto	**XC88228**, XC124364, XC88226, XC88229
Heteromirafra ruddi	Rudd’s Lark	Per Alström	**PA_2017-1056**, PA_2017-1061, PA_2017-1099, PA_2017-1105
Calendulauda barlowi	Barlow’s Lark	Xeno-canto	**XC158041**, XC146425, XC146426
		Per Alström	PA_1010
Calendulauda erythrochlamys	Dune Lark	Xeno-canto	**XC58517**
		AvoCet	AV_08-0-22-1, AV_08-0-22-2

	Calendulauda albescens	Karoo Lark	Xeno-canto	**XC58017,** XC58018
			Per Alström	PA_1051, PA_1045
	Calendulauda africanoides	Fawn coloured Lark	Xeno-canto	**XC58708,** XC28606
			Per Alström	PA_1020, PA_1021-1, PA_1021-2
	Calendulauda alopex	Foxy Lark	Xeno-canto	**XC300042**, XC300041, XC300043
			MLNS	ML21270
	Calendulauda burra	Red Lark	Xeno-canto	**XC146481**
			Per Alström	PA_1009, PA_1035
	Calendulauda sabota	Sabota Lark	Per Alström	**PA_2017-1090-1**, PA_2017-1090-2, PA_2017-1095
			AvoCet	AV_O-67-1
Clade C - Ammomanid	*Eremopterix leucotis*	Chestnut-backed Sparrow-Lark	Xeno-canto	**ML61186**
	Eremopterix griseus	Ashy-crowned Sparrow-Lark	Xeno-canto	**XC86569**
	Eremopterix signatus	Chestnut-headed Sparrow-Lark	Xeno-canto	**XC209981**
	Eremopterix verticalis	Grey-backed Sparrow Lark	MLNS	**ML61158**
	Eremopterix nigriceps	Black-crowned Sparrow-Lark	Xeno-canto	**XC355815**, XC35816
	Ammomanes cintura	Bar-tailed Lark	Xeno-canto	**XC131914**, XC131913
	Ammomanes deserti	Desert Lark	MLNS	**ML1723**
			Xeno-canto	XC44494
	Ramphocoris clotbey	Thick-billed Lark	Xeno-canto	**XC134442**
	Certhilauda brevirostris	Agulhas long-billed Lark	Xeno-canto	**XC288868**, XC62564, XC62562 XC62563
	Certhilauda curvirostris	Cape long-billed Lark	Xeno-canto	**XC278147**, XC62554
			Per Alström	PA_1011, PA_1017, PA_1039
	Certhilauda semitorquata	Eastern long-billed Lark	Xeno-canto	**XC216682,** XC233912, XC279991
			Per Alström	PA_2017-1065, PA_2017-1070

Certhilauda benguelensis	Benguela long-billed Lark	Xeno-canto	**XC65245**
Certhilauda subcoronata	Karoo long-billed Lark	Per Alström	**PA_1030**
		Xeno-canto	XC146478, XC126492
		AvoCet	AV1-0-57-1
Certhilauda chuana	Short clawed Lark	Per Alström	**PA_93-01-30**
Chersomanes albofasciata	Spike-heeled Lark	Xeno-canto	**XC126330**, XC204237
Ammomanopsis grayi	Gray's Lark	MLNS	**ML61100**, ML61107
		Xeno-canto	XC65276
Alaemon alaudipes	Greater Hoopoe-Lark	Xeno-canto	**XC164131**, XC135148

TABLE 3.2. LIST OF LARK SPECIES, SPECTROGRAM AND AURAL CHARACTERISTICS GENERATED FROM SONG STROPHES OF EACH SPECIES.

Clades	Scientific name	Strophe length	General strophe pitch	Strophe type (aurally)	Grouped element-ending	Grouped element-ending structure	Wing clapping	Wing clapping incorporation in song	Mimicry
		short (≤4 s)	descending	predominantly tonal/musical	absent	not applicable	absent	not applicable	unknown
		intermediate (4.1s – 8 s)	ascending	predominantly harsh/screeching	present	warbling/bubbling	present	absent	known
		long (>8 s)	stable			trilling		present	
A	*Galerida magnirostris*	short	ascending	musical	present	warbling/bubbling	absent	not applicable	known
	Galerida deva	short	descending	musical	absent	not applicable	absent	not applicable	known
	Galerida theklae	short	descending	musical	present	warbling/bubbling	absent	not applicable	known
	Galerida malabarica	short	ascending	musical	absent	not applicable	absent	not applicable	known
	Galerida cristata	short	ascending	musical	present	trilling	absent	not applicable	known
	Galerida macrorhyncha	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Calandrella cinerea	short	descending	musical	absent	not applicable	absent	not applicable	known
	Calandrella acutirostris	short	ascending	musical	absent	not applicable	absent	not applicable	known
	Calandrella erlanger	short	descending	musical	absent	not applicable	absent	not applicable	known
	Calandrella brachydactyla	short	descending	musical	absent	not applicable	absent	not applicable	known
	Eremophila bilopha	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Eremophila alpestris	short	ascending	musical	present	warbling/bubbling	absent	not applicable	unknown
	Alauda leucoptera	short	descending	musical	absent	not applicable	absent	not applicable	known
	Chersophilus duponti	intermediate	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Alaudala cheleensis	long	ascending	musical	present	warbling/bubbling	absent	not applicable	known
	Alaudala rufescens	long	ascending	musical	present	warbling/bubbling	absent	not applicable	known
	Alaudala somalica	intermediate	descending	harsh/screeching	absent	not applicable	absent	not applicable	known

	Alaudala raytal	intermediate	descending	musical	present	warbling/bubbling	absent	not applicable	known
	Melanocorypha maxima	long	descending	musical	present	trilling	absent	not applicable	known
	Melanocorypha bimaculata	short	ascending	harsh/screeching	absent	not applicable	absent	not applicable	known
	Melanocorypha yeltoniensis	short	descending	musical	present	warbling/bubbling	absent	not applicable	unknown
	Melanocorypha calandra	intermediate	ascending	musical	present	trilling	absent	not applicable	known
	Spizocorys conirostris	short	stable	musical	absent	not applicable	absent	not applicable	unknown
	Spizocorys fringillaris	short	stable	musical	absent	not applicable	absent	not applicable	unknown
	Spizocorys sclateri	short	stable	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Spizocorys starki	short	stable	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Lullula arborea	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
B	*Mirafra africana*	short	descending	musical	absent	not applicable	present	absent	known
	Mirafra angolensis	intermediate	ascending	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Mirafra cheniana	short	stable	musical	absent	not applicable	absent	not applicable	known
	Mirafra gilletti	short	descending	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Mirafra pulpa	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Mirafra hypermetra	short	ascending	musical	absent	not applicable	absent	not applicable	known
	Mirafra passerina	short	ascending	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Mirafra rufocinnamomea	short	stable	musical	absent	not applicable	present	absent	known
	Mirafra fasciolata	short	ascending	musical	absent	not applicable	present	present	known
	Mirafra apiata	short	ascending	musical	absent	not applicable	present	present	known
	Mirafra javanica	short	ascending	musical	present	warbling/bubbling	absent	not applicable	known
	Mirafra microptera	short	ascending	musical	present	warbling/bubbling	absent	not applicable	unknown
	Mirafra assamica	short	ascending	harsh/screeching	absent	not applicable	absent	not applicable	known
	Mirafra erythrocephala	intermediate	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Mirafra affinis	intermediate	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Mirafra cantillans	intermediate	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Mirafra erythroptera	intermediate	ascending	musical	absent	not applicable	absent	not applicable	unknown

	Heteromirafra ruddi	short	stable	musical	absent	not applicable	absent	not applicable	unknown
	Calendulauda barlowi	short	ascending	musical	present	trilling	absent	not applicable	unknown
	Calendulauda erythrochlamys	short	ascending	musical	present	trilling	absent	not applicable	unknown
	Calendulauda albescens	short	ascending	musical	present	trilling	absent	not applicable	unknown
	Calendulauda burra	short	descending	musical	present	trilling	absent	not applicable	unknown
	Calendulauda africanoides	short	descending	musical	absent	not applicable	absent	not applicable	known
	Calendulauda alopex	short	ascending	musical	absent	not applicable	absent	not applicable	known
	Calendulauda sabota	short	descending	musical	absent	not applicable	absent	not applicable	known
C	*Certhilauda brevirostris*	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Certhilauda curvirostris	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Certhilauda semitorquata	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Certhilauda benguelensis	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Certhilauda subcoronata	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Certhilauda chuana	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Eremopterix nigriceps	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Eremopterix griseus	short	ascending	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Eremopterix signatus	short	descending	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Eremopterix leucotis	short	ascending	musical	absent	not applicable	absent	not applicable	known
	Eremopterix verticalis	intermediate	descending	harsh/screeching	absent	not applicable	absent	not applicable	unknown
	Ammomanes cintura	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Ammomanes deserti	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Ramphocorys clotbey	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Ammomanopsis grayi	short	ascending	musical	absent	not applicable	absent	not applicable	unknown
	Chersomanes albofasciata	short	descending	musical	absent	not applicable	absent	not applicable	unknown
	Alaemon alaudipes	Long	ascending	musical	absent	not applicable	absent	not applicable	unknown

TABLE 3.3. THE MEAN AND STANDARD DEVIATION FOR EACH VARIABLE ACROSS ALL CLADES, INCLUDING THE *F*-STATISTIC AND *P*-VALUE FROM THE ANOVA AT CLADE LEVEL. AN ASTERISK INDICATES SIGNIFICANCE AT THE $P = 0.05$ LEVEL. A HEREAFTER REPRESENTS THE ALAUDID CLADE, B – MIRAFRID CLADE AND C – AMMOMANID CLADE.

Song strophe Variables	Clade			Clade level ANOVA	
	A	B	C	F *statistic*	*P*-value
F_{max} (kHz)	5659 ± 778.3	5411 ± 1190	4782 ± 1277	4.426	0.0157*
F_{min} (kHz)	2597 ± 797.7	3010 ± 1334	2797 ± 1085	0.568	0.569
F_{band} (kHz)	3062± 1106.3	2401 ± 1111	1986 ± 1501	5.921	0.00431*
F_{peak} (kHz)	4890 ± 789.8	4788 ± 1324	4103 ± 1154	4.416	0.0159*
N_{ele}	8.7 ± 3.9	10.9 ± 12.9	6.2 ± 5.6	1.829	0.169
S_{dur} (s)	3.4 ± 2.9	2.7 ± 1.8	2.7 ± 2.7	0.691	0.505

TABLE 3.4. THE VARIABLES EXTRACTED BY PRINCIPAL COMPONENT ANALYSIS (PCA) WITH THE FIRST TWO PC SCORES FOR THE CLADE. EIGENVALUES AND PERCENTAGE VARIANCE EACH COMPONENT EXPLAIN IN THE DATA ARE SHOWN. LOADINGS (|0.5|) ARE INDICATED BY AN ASTERISK.

Song Variables	Clade level (PCA)	
	PC 1	PC 2
F_{max}	0.94*	-0.06
F_{band}	0.74*	0.66*
F_{peak}	0.84*	-0.51*
F_{min}	-	-
N_{ele}	-	-
S_{dur} (s)	-	-
Eigenvalue	1.47	0.84
% Variance explained	72.24	23.33

TABLE 3.5. *POST-HOC* TUKEY'S HONESTLY SIGNIFICANT DIFFERENCE (HSD) TEST FOR THE TWO PRINCIPAL COMPONENT (PC) SCORES SIGNIFICANTLY DIFFER AT CLADE LEVEL.

Principal components	Clade level
PC 1	Clade A – Clade C ($P < 0.01$)
PC 2	-

TABLE 3.6. DISCRIMINANT FUNCTION ANALYSIS (DFA) PREDICTION OF SPECIES MEMBERSHIP IN THE THREE CLADES (A, B AND C). SUMMATION OF SPECIES IS VERTICAL. ASTERISK (*) INDICATES THE CORRECT CLASSIFICATION.

Clade Predicted	A	B	C
A	**19***	11	5
B	7	**10***	3
C	1	4	**9***

TABLE 3.7. THE *F*-STATISTIC AND *P*-VALUES FROM ANALYSIS OF VARIANCE (ANOVA) FOR ALL THE VARIABLE USED WITHIN EACH CLADE. AN ASTERISK INDICATES SIGNIFICANCE AT 0.05 LEVEL.

Song Variable	Clade A		Clade B		Clade C	
	F *statistic*	*P*-value	F *statistic*	*P*-value	F *statistic*	*P*-value
F_{max} (kHz)	5.711	0.00105*	4.722	0.0114*	0.703	0.654
F_{min} (kHz)	0.872	0.557	9.649	0.000333*	0.401	0.862
F_{band} (kHz)	1.262	0.322	2	0.145	0.558	0.754
F_{peak} (kHz)	2.839	0.0314*	7.08	0.00182*	0.682	0.669
N_{ele}	2.636	0.0418*	0.535	0.593	15.38	0.000157*
S_{dur} (s)	2.603	0.0438*	3.177	0.0453*	22.53	0.0000218*

TABLE 3.8. *POST-HOC* TUKEY'S HSD TEST FOR ANOVA SHOWING SIGNIFICANT DIFFERENCES BETWEEN GENERA ACROSS ALL THE THREE CLADES (A, B AND C).

Variable Significant	Clade A	Clade B	Clade C
F_{max}	*Galerida* – *Alaudala* (*P* < 0.01), *Galerida* – *Melanocorypha* (*P* < 0.01)	*MirafraN* – *MirafraS* (*P* < 0.05)	-
F_{min}	-	*MirafraN* - *MirafraS* (*P* < 0.01) *MirafraN* - *Heteromirafra* (*P* < 0.01) *MirafraN* – *Calendulauda* (*P* < 0.05)	-
F_{peak}	*Alaudala* – *Spizocorys* (*P* < 0.05)	*MirafraN* – *MirafraS* (*P* < 0.01) *MirafraS* – *Calendulauda* (*P* < 0.05)	-
N_{ele}	*Alaudala* – *Spizocorys* (*P* < 0.05)	-	*Certhilauda* – *Eremopterix* (*P* < 0.01) *Certhilauda* – *Ammomanopsis* (*P* < 0.01) *Certhilauda* – *Chersomanes* (*P* < 0.01) *Certhilauda* – *Alaemon* (*P* < 0.01) *Alaemon* – *Eremopterix* (*P* < 0.05) *Ammomanopsis* – *Ammomanes* (*P* < 0.05) *Chersomanes* – *Ammomanes* (*P* < 0.05) *Alaemon* – *Ammomanes* (*P* < 0.01) *Alaemon* – *Ramphocorys* (*P* < 0.05)
S_{dur}	*Lullula* – *Spizocorys* (*P* < 0.05)	-	*Alaemon* – *Certhilauda* (*P* < 0.01) *Alaemon* – *Eremopterix* (*P* < 0.01) *Alaemon* – *Ammomanes* (*P* < 0.01) *Alaemon* – *Ramphocorys* (*P* < 0.01) *Alaemon* – *Ammomanopsis* (*P* < 0.01) *Alaemon* – *Chersomanes* (*P* < 0.01)

TABLE 3.9. THE VARIABLES EXTRACTED BY PRINCIPAL COMPONENT ANALYSIS (PCA) WITH THE FIRST TWO PRINCIPAL COMPONENTS (PC) SCORES FOR CLADE A, CLADE B AND CLADE C, RESPECTIVELY. EIGENVALUES AND PERCENTAGE VARIANCE EACH COMPONENT EXPLAIN IN THE DATA WITHIN EACH CLADE ARE SHOWN. LOADINGS (|0.5|) ARE INDICATED BY ASTERISK.

Song Variables	Clade A (PCA)		Clade B (PCA)		Clade C (PCA)	
	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
F_{max}	0.83*	-0.44	0.90*	-0.22	0.51*	0.72*
F_{min}	-	-	0.78*	-0.42	-	-
F_{band}	-	-	-	-	-	-
F_{peak}	0.93*	-0.21	0.95*	-0.10	0.67*	0.54*
N_{ele}	0.48	0.80*	-	-	0.92*	-0.29
S_{dur}	0.72*	0.24	0.74*	0.63*	0.73*	-0.63*
Eigenvalue	1.52	0.97	1.70	0.76	1.44	1.14
%Variance explained	57.53	23.33	71.92	14.55	51.98	32.25

TABLE 3.10. *POST-HOC* TUKEY'S HONESTLY SIGNIFICANT DIFFERENCE (HSD) TEST FOR PRINCIPAL COMPONENT (PC) 1 AND PC2 SHOWING SIGNIFICANT DIFFERENCES BETWEEN GENERA ACROSS CLADE A, CLADE B AND CLADE C.

Principal component	Clade A	Clade B	Clade C
PC 1	*Alaudala* – *Galerida* ($P < 0.05$), *Alaudala* – *Spizocorys* ($P < 0.05$)	*MirafraN* – *MirafraS* ($P < 0.001$), *MirafraN* – *Heteromirafra* ($P < 0.05$)	*Alaemon* – *Certhilauda* ($P < 0.05$)
PC 2	*Galerida* – *Melanocorypha* ($P < 0.05$), *Galerida* – *Spizocorys* ($P < 0.05$), *Lullula* – *Eremophila* ($P < 0.05$), *Lullula* – *Alauda* ($P < 0.05$), *Lullula* – *Melanocorypha* ($P < 0.01$), *Lullula* – *Spizocorys* ($P < 0.01$)	-	-

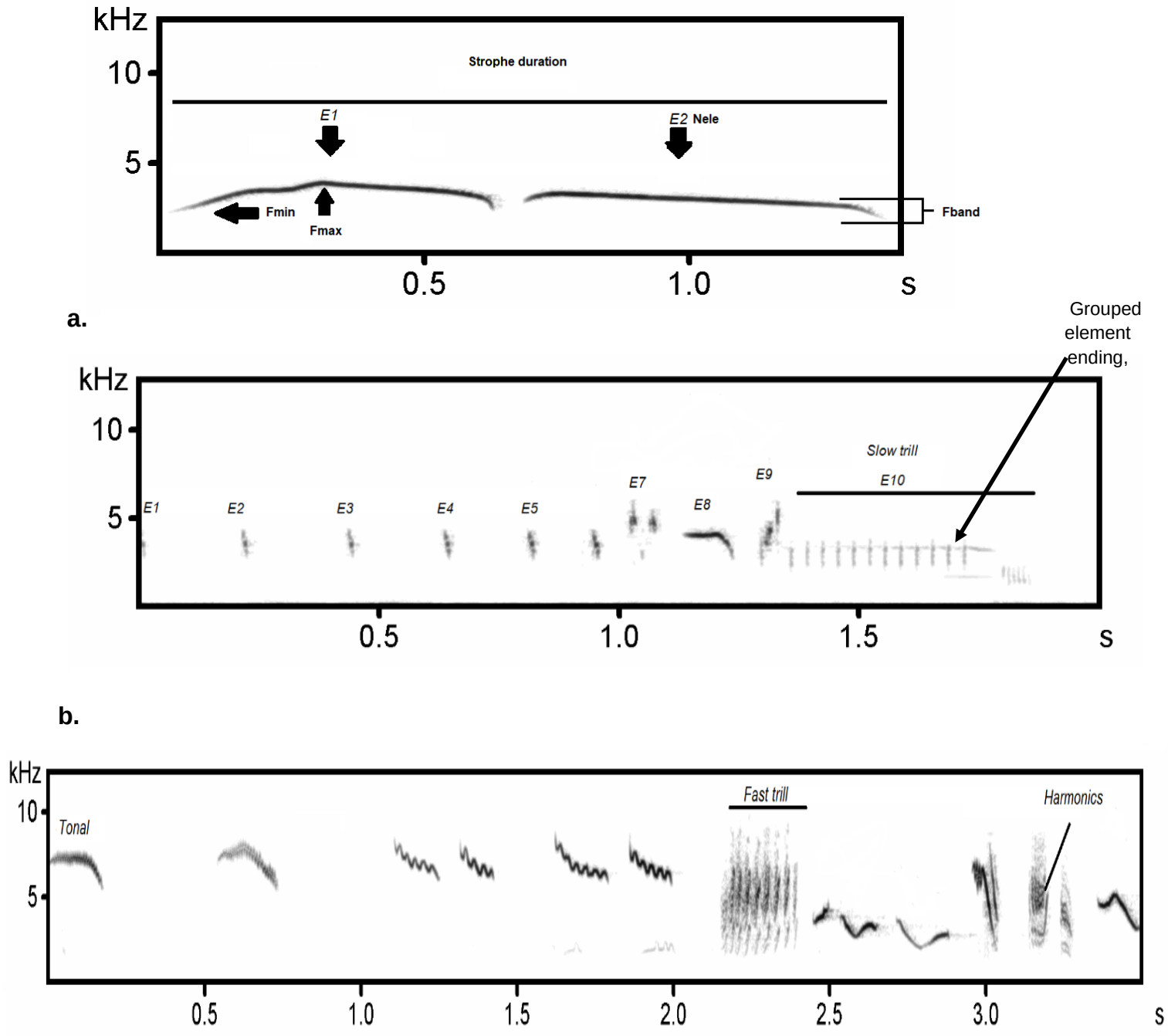


FIGURE 3.1. EXAMPLES OF SPECTROGRAMS OF SONG STROPHES OF LARKS RANGING FROM SIMPLE (A. AGULHAS LONG-BILLED LARK *CERTHILAUDA BREVIROSTRIS*), LESS COMPLEX (B. DUNE LARK *CALENDULAUDA ERYTHROCHLAMYS*) AND COMPLEX (C. SABOTA LARK *CALENDULAUDA SABOTA*). E – ELEMENT, E1 – ELEMENT NUMBER 1, N_{ELE} – NUMBER OF ELEMENTS, F_{MIN} – MINIMUM FREQUENCY, F_{MAX} – MAXIMUM FREQUENCY, F_{BAND} – BANDWIDTH FREQUENCY.

CLADE LEVEL – CLADE A (ALAUDID), CLADE B (MIRAFRID) AND CLADE C (AMMOMANID)TOGETHER

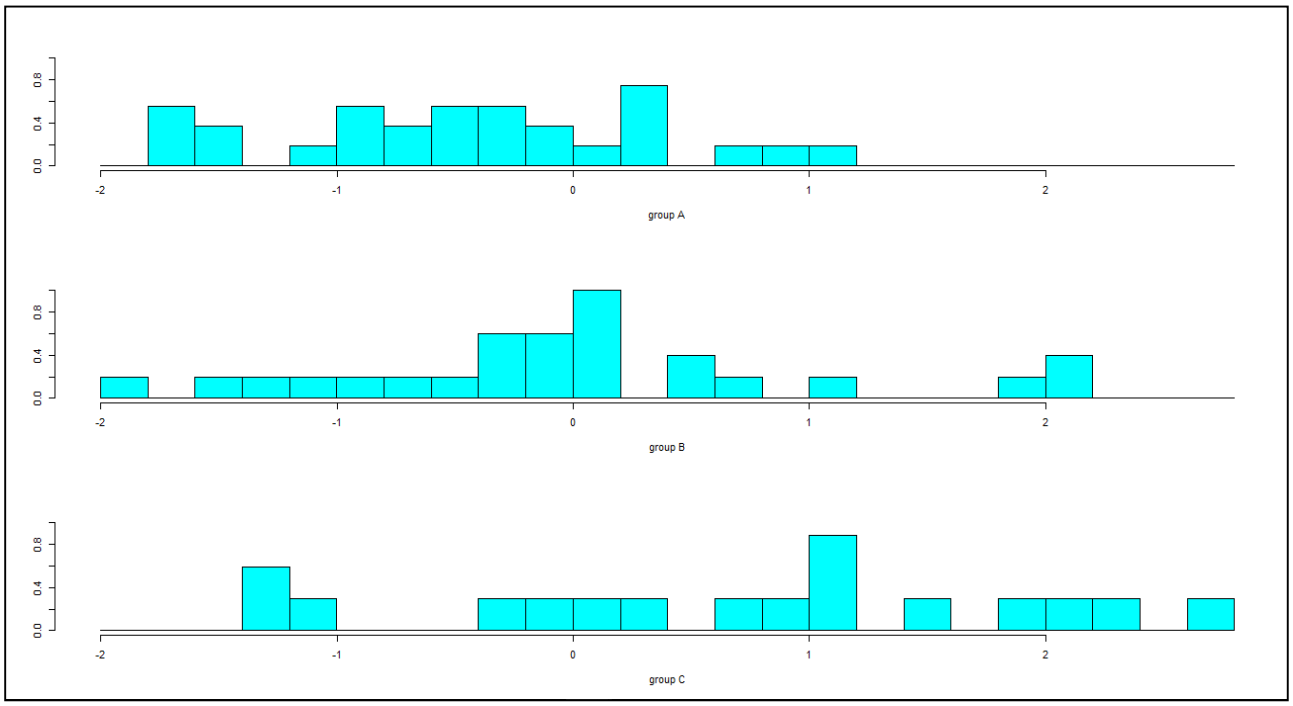


FIGURE 3.2. SCATTERPLOT SHOWING A GREATER OVERLAP BETWEEN CLADE (GROUP) A AND CLADE B THAN BETWEEN BOTH A AND B AND CLADE C.

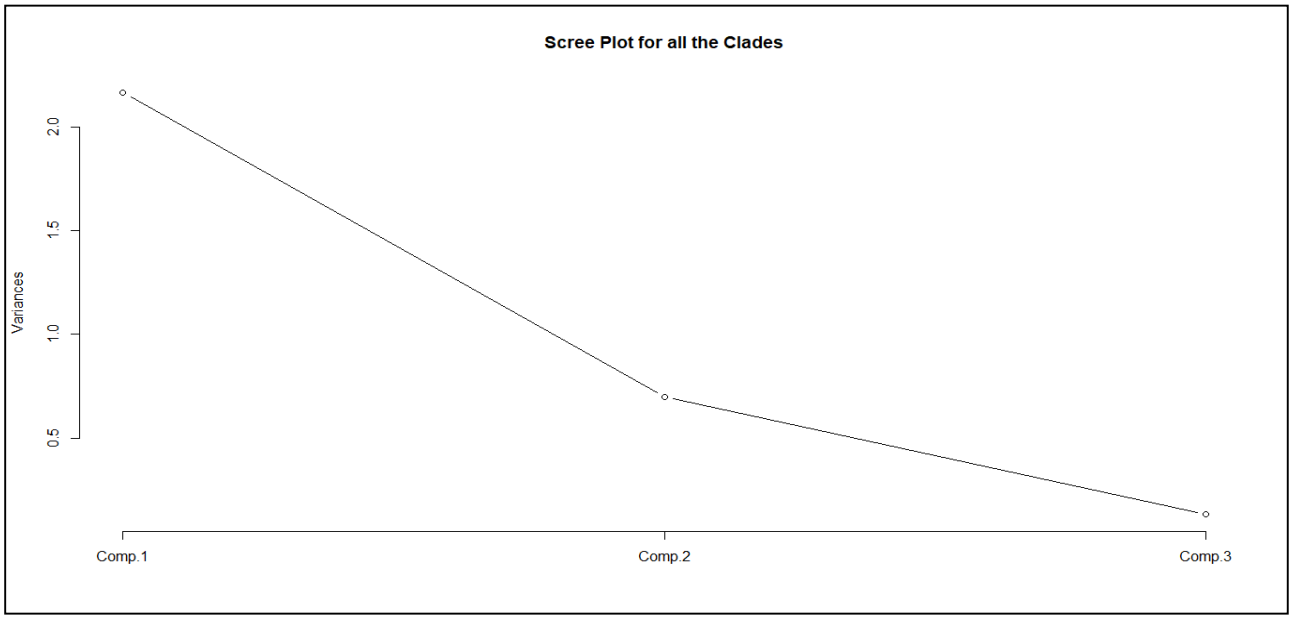


FIGURE 3.3. SCREE PLOT SHOWING THE EIGENVALUES AND COMPONENTS EXTRACTED RELATIVE TO THE VARIANCE OF THE THREE SIGNIFICANTLY DIFFERENT VARIABLES (F_{MAX}, F_{PEAK} AND F_{BAND}) USED IN PRINCIPAL COMPONENT ANALYSIS AT CLADE LEVEL.

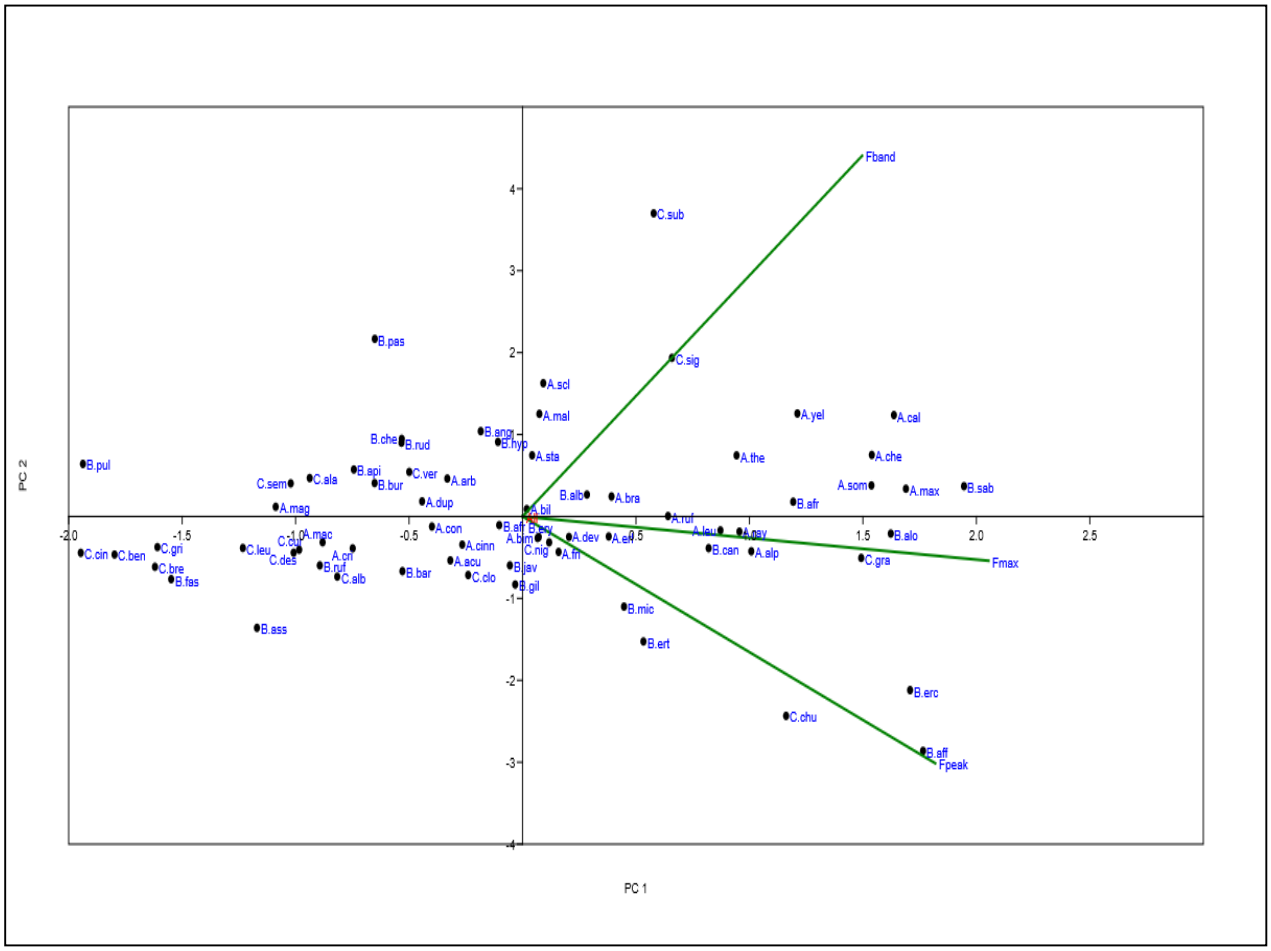


FIGURE 3.4. BIPLOT OF PRINCIPAL COMPONENT (PC) 1 AND PC2 SCORES FROM PRINCIPAL COMPONENT ANALYSIS (PCA) FROM THE THREE SIGNIFICANTLY DIFFERENT VARIABLES (F_{MAX}, F_{PEAK} AND F_{BAND}) AT CLADE LEVEL. IN THE ABBREVIATION C.CH, C STANDS FOR CLADE C, CHU REPRESENTS THE SPECIFIC EPITHET '*CHUANA*'. REFER TO APPENDIX 3.1 FOR ALL SPECIES NAMES.

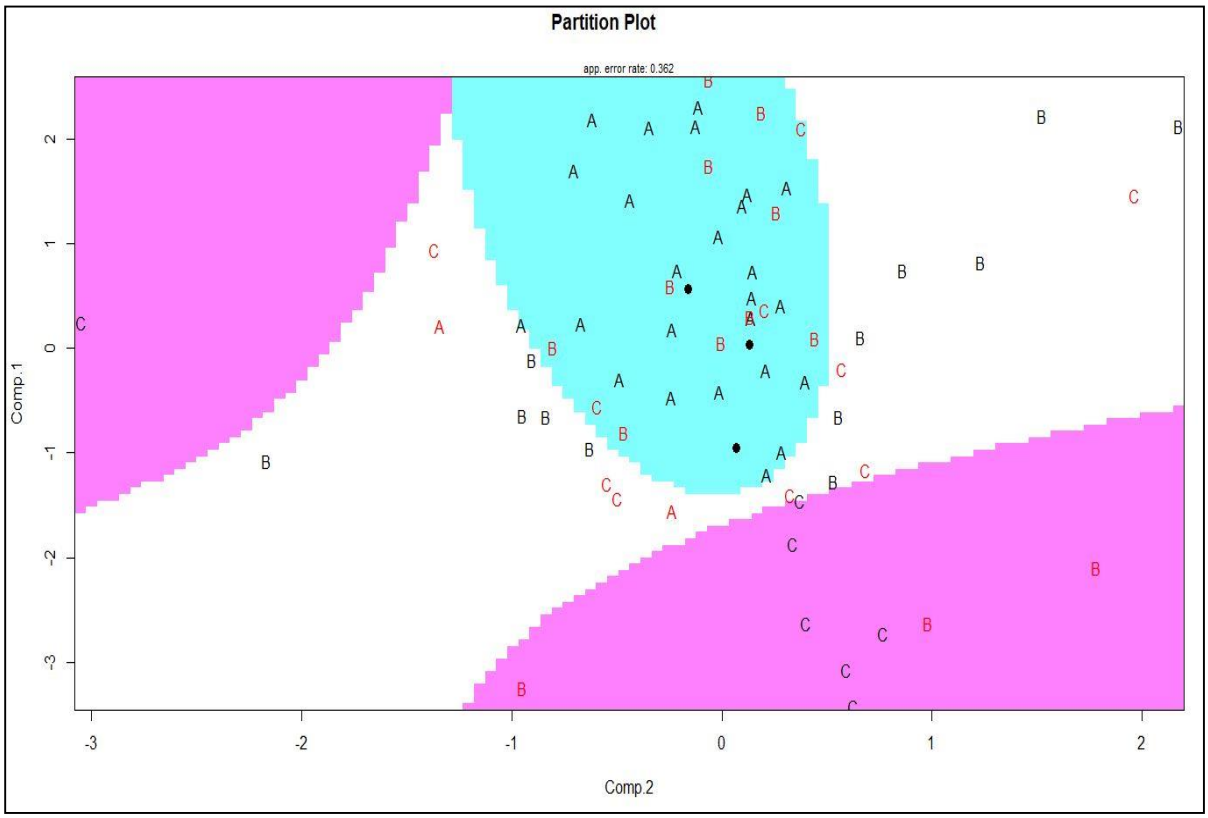


FIGURE 3.5. PARTITION PLOT FROM DISCRIMINANT FUNCTION ANALYSIS (DFA) BASED ON SIGNIFICANTLY DIFFERENT VARIABLES SHOWING THE SEPARATION OF THE THREE MAJOR CLADES BASED ON PC1 AND PC2. ALPHABETS IN BLACK COLOUR SHOW THE CORRECT GROUPING OF EACH OF THE CLADES (A, B, C) AND RED ALPHABETS REPRESENT CLADES THAT ARE INCORRECTLY GROUPED. THE PINK SHADE IS CLADE C CLUSTER, BLUE SHADE IS CLADE A CLUSTER AND WHITE SHADE IS CLADE B CLUSTER. REFER TO APPENDIX 3.1 FOR SPECIES NAMES.

CLADE A (ALAUDID) ONLY

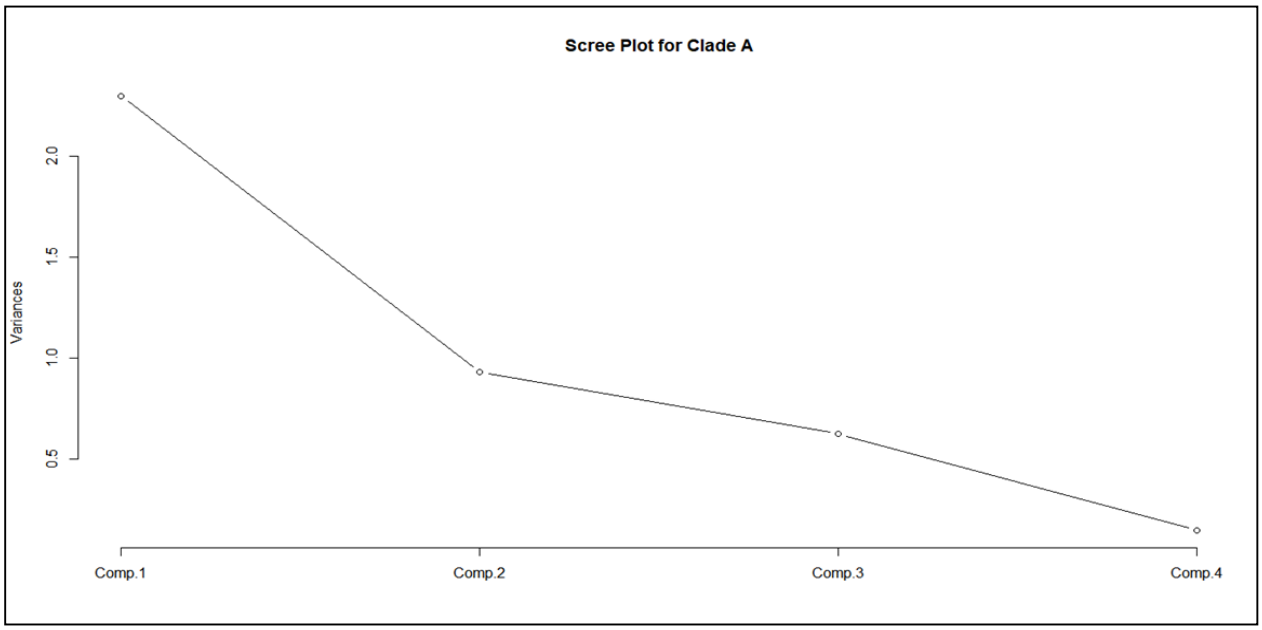


FIGURE 3.6. SCREE PLOT SHOWING THE EIGENVALUES PERTAINING TO VARIANCE FOR EACH COMPONENT EXTRACTED. FOUR VARIABLES (F_{MAX}, F_{PEAK}, N_{ELE} AND S_{DUR}) WERE USED IN PRINCIPAL COMPONENT ANALYSIS (PCA) FOR CLADE A.

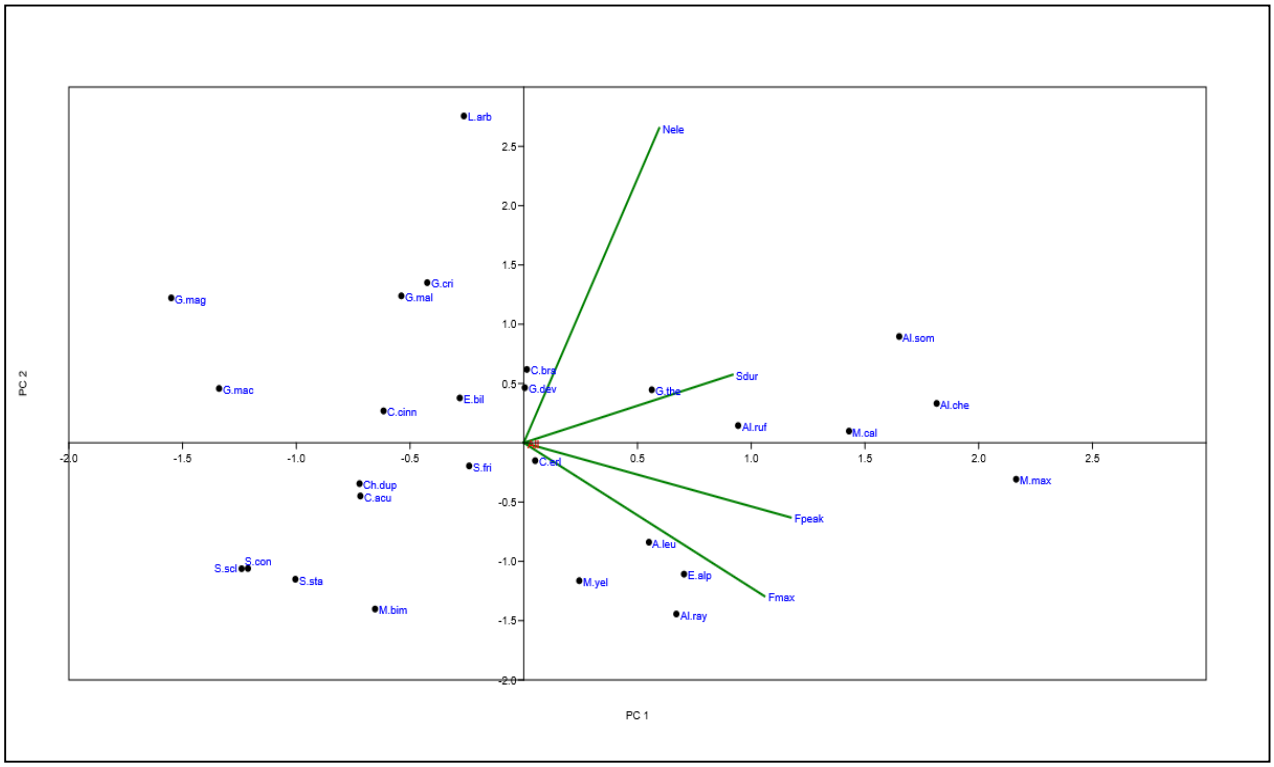


FIGURE 3.7. BIPLOT OF PRINCIPAL COMPONENT 1 (PC1) AND PC2 SCORES FROM PRINCIPAL COMPONENT ANALYSIS (PCA) OF ALL FOUR VARIABLES (F_{MAX}, F_{PEAK}, N_{ELE} AND S_{DUR}) AT CLADE A. IN THE ABBREVIATION C.ACU, C STANDS FOR GENUS *CALANDRELLA*, ACU REPRESENTS THE SPECIFIC EPITHET '*ACUTIROSTRIS*'. REFER TO APPENDIX 3.1 FOR SPECIES NAMES.

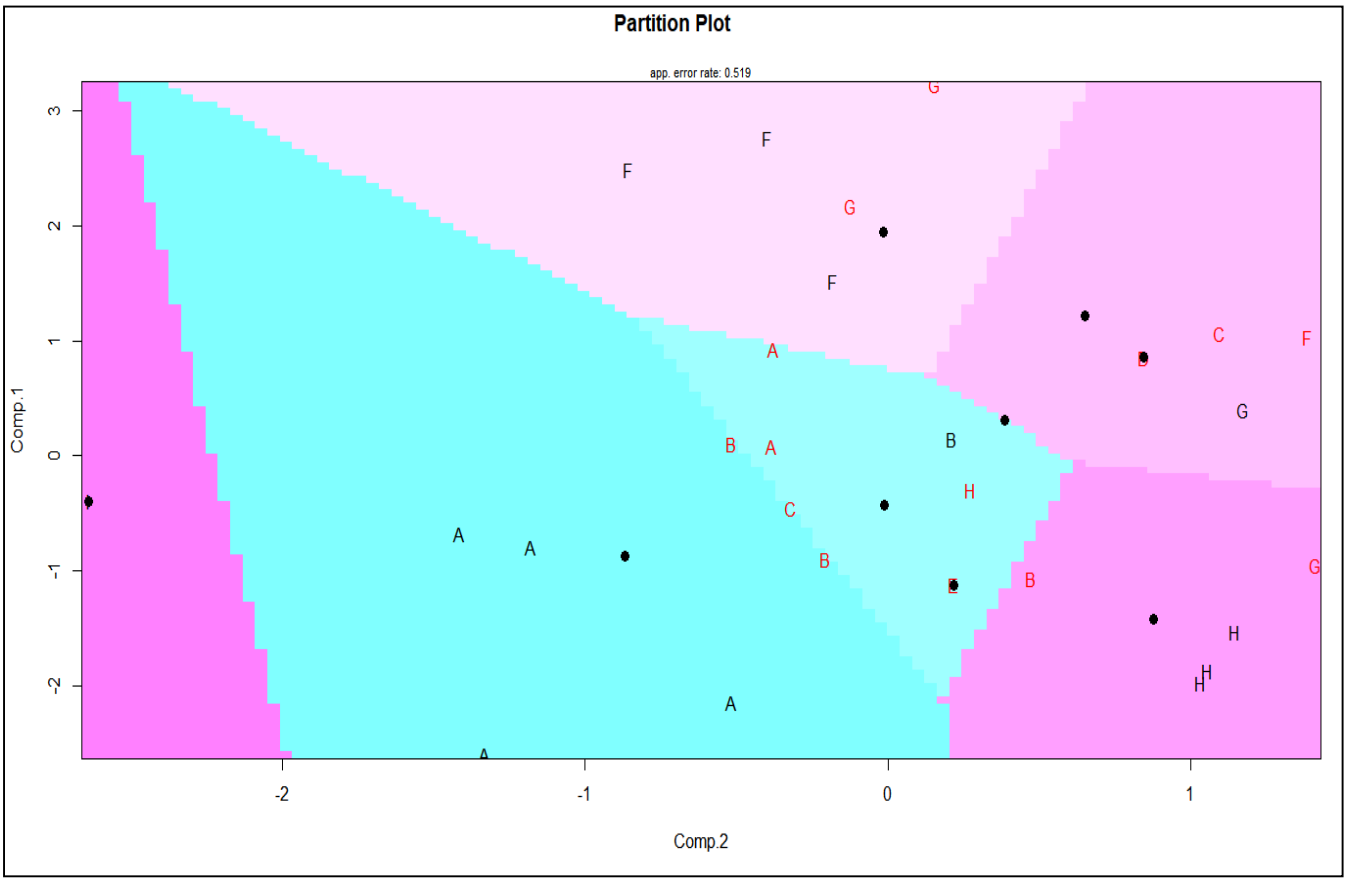


FIGURE 3.8. PARTITION PLOT FROM DISCRIMINANT FUNCTION ANALYSIS (DFA) AT CLADE A BASED ON PC1 AND PC2. REFER TO APPENDIX 3.1 FOR SPECIES NAMES. A = *GALERIDA*, B = *CALANDRELLA*, C = *EREMOPHILA*, D = *ALAUDA*, E= *CHERSOPHILUS*, F = *ALAUDALA*, G = *MELANOCORYPHA*, H = *SPIZOCORYS*, I = *LULLULA*.

CLADE B (MIRAFRID) ONLY

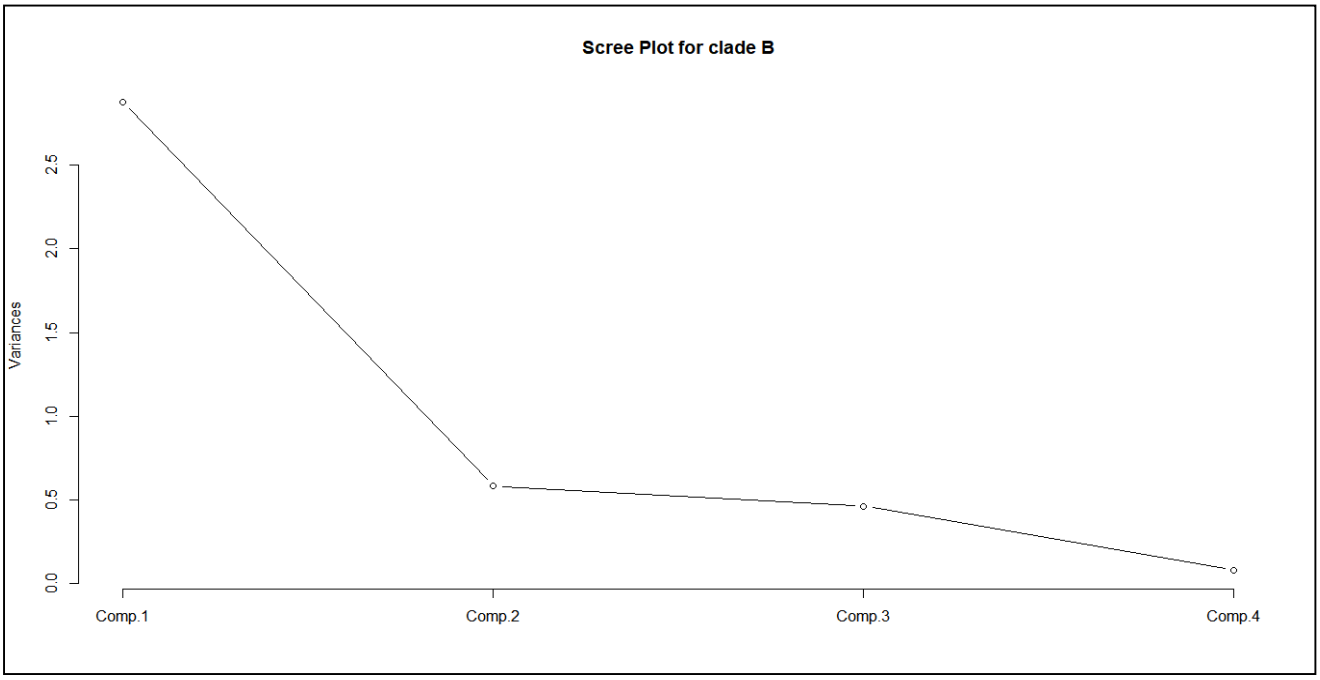


FIGURE 3.9. SCREE PLOT SHOWING THE EIGENVALUES PERTAINING TO VARIANCE FOR EACH COMPONENT EXTRACTED. FOUR VARIABLES (F_{MAX}, F_{MIN}, F_{PEAK} AND S_{DUR}) USED IN PRINCIPAL COMPONENT ANALYSIS (PCA) FOR CLADE B.

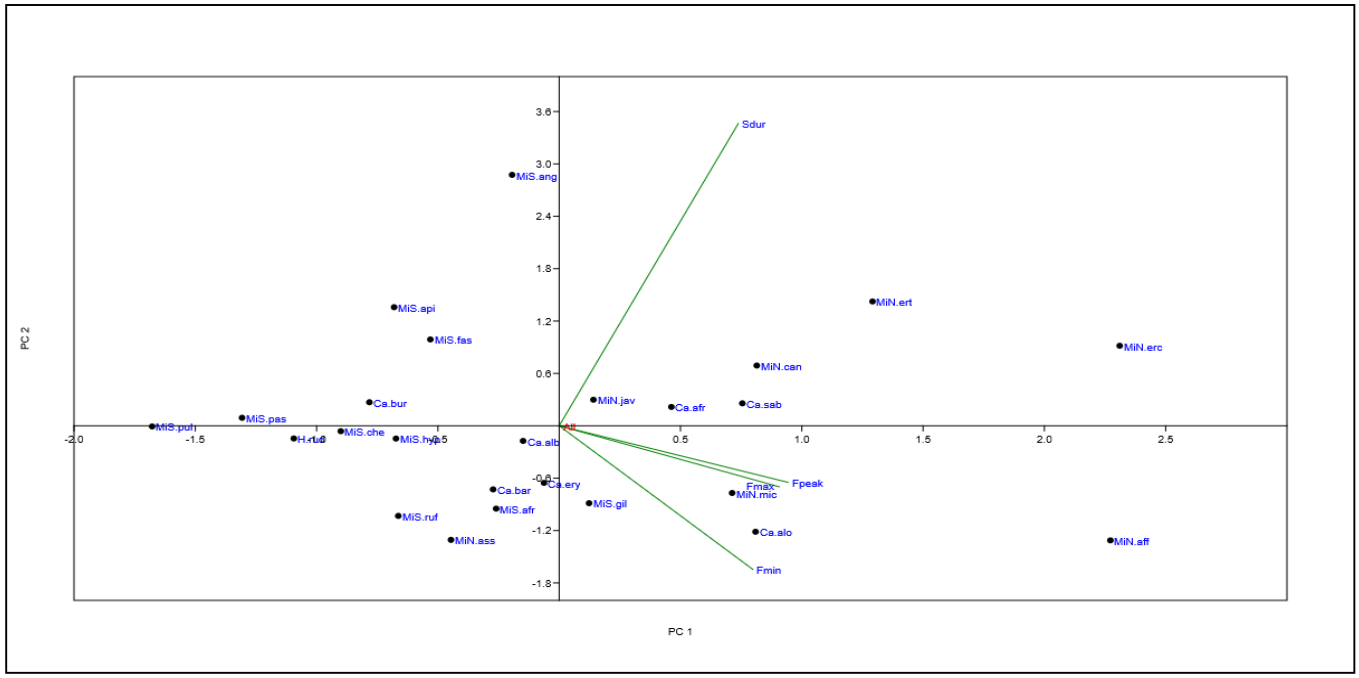


FIGURE 3.10. BIPLOT OF PRINCIPAL COMPONENT 1 (PC1) AND PC2 SCORES FROM PRINCIPAL COMPONENT ANALYSIS (PCA) FROM FOUR VARIABLES (F_{MAX}, F_{MIN}, F_{PEAK} AND S_{DUR}) AT CLADE B. IN THE ABBREVIATION CA.AFRI, CA STANDS FOR GENUS *CALENDULAUDA*, AFRI REPRESENTS THE SPECIFIC EPITHET '*AFRICANOIDES*'. REFER TO APPENDIX 3.1 FOR SPECIES NAMES.

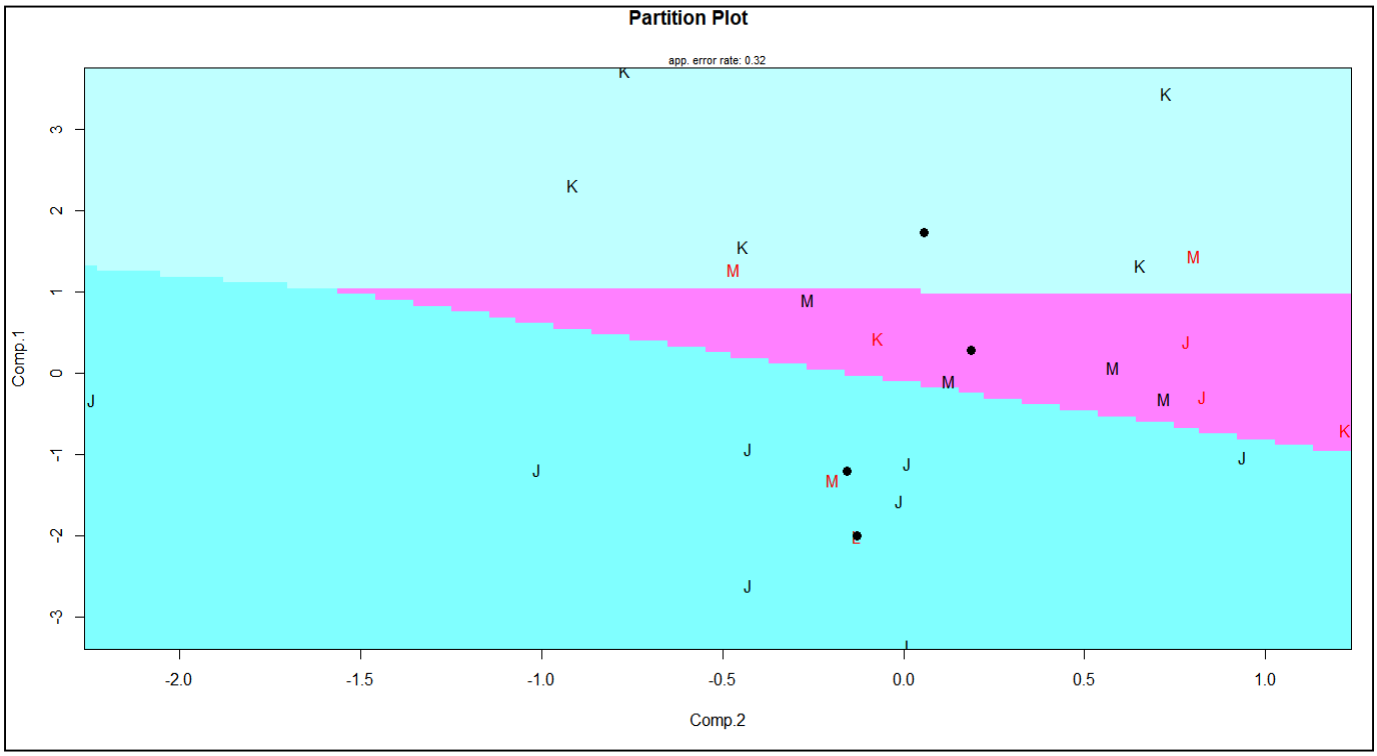


FIGURE 3.11. PARTITION PLOT FROM DISCRIMINANT FUNCTION ANALYSIS (DFA) AT CLADE B BASED ON PC1 AND PC2. REFER TO APPENDIX 3.1 FOR THE SPECIES NAME. J = *MIRAFRA* SOUTHERN, K = *MIRAFRA* NORTHERN, L = *HETEROMIRAFRA*, M = *CALENDULAUDA*.

CLADE C (AMMOMANID) ONLY

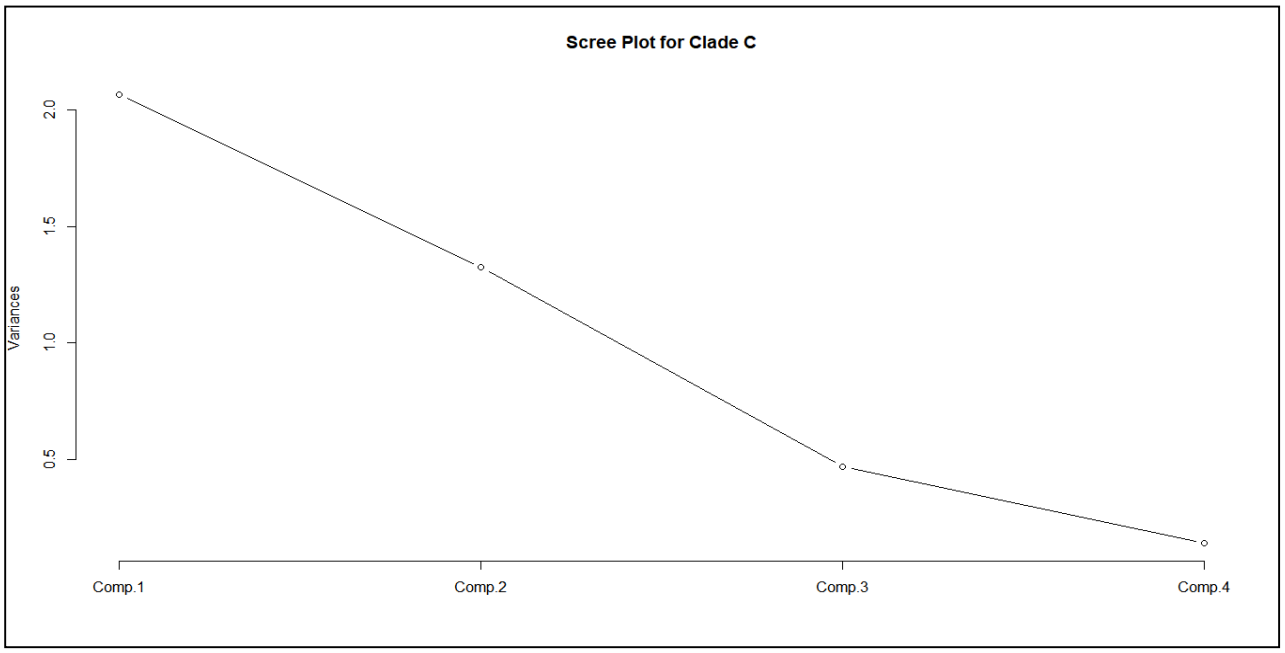


FIGURE 3.12. SCREE PLOT SHOWING THE EIGENVALUES PERTAINING TO VARIANCE FOR EACH COMPONENT EXTRACTED. FOUR VARIABLES (F_{MAX}, N_{ELE}, F_{PEAK} AND S_{DUR}) USED IN PRINCIPAL COMPONENT ANALYSIS (PCA) FOR CLADE C.

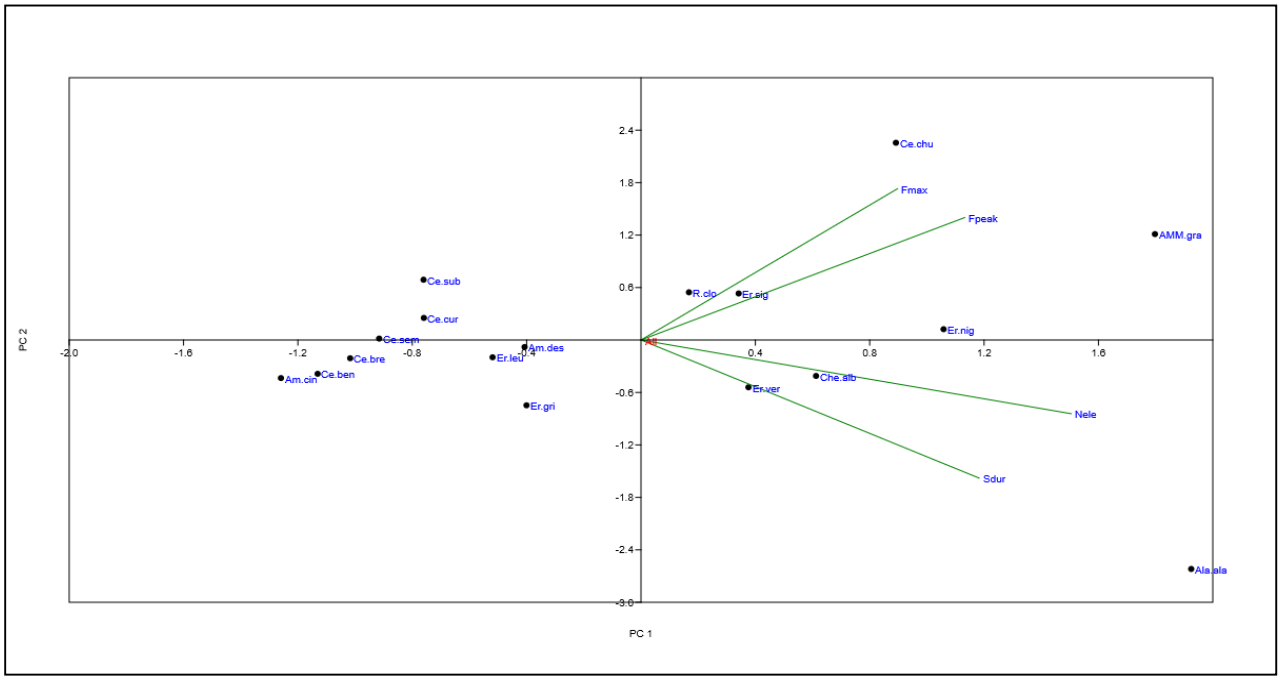


FIGURE 3.13. BIPLOT OF PRINCIPAL COMPONENT 1 (PC1) AND PC2 SCORES FROM PRINCIPAL COMPONENT ANALYSIS (PCA) FROM FOUR VARIABLES (F_{MAX}, N_{ELE}, F_{PEAK} AND S_{DUR}) AT CLADE C. IN THE ABBREVIATION ER.NIG, ER STANDS FOR GENUS *EREMOPTERIX*, NIG REPRESENTS THE SPECIFIC EPITHET '*NIGRICEPS*'. REFER TO APPENDIX 3.1 FOR SPECIES NAMES.

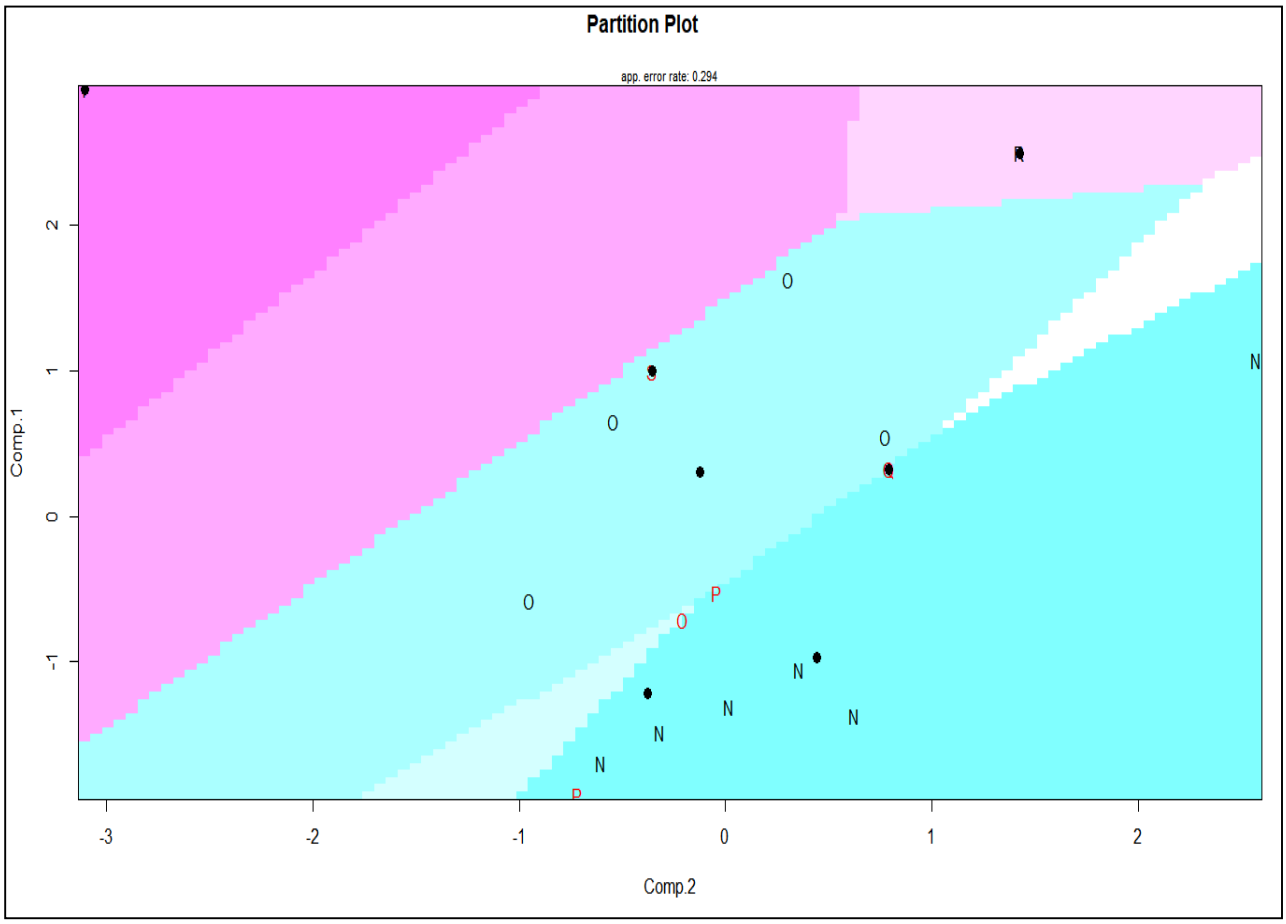


FIGURE 3.14. PARTITION PLOT FROM DISCRIMINANT FUNCTION ANALYSIS (DFA) AT CLADE C BASED ON PC1 AND PC2. REFER TO APPENDIX 3.1 FOR SPECIES NAMES. N = *CERTHILAUDA*, O = *EREMOPTERIX*, P = *AMMOMANES*, Q = *RAMPHOCORYS*, R = *AMMOMANOPSIS*, S = *CHERSOMANES*, T = *ALAEMON*.

APPENDIX 3.1. LIST OF SPECIES USED IN CHAPTER 3 INCLUDING THE CODES USED IN THE BIPLOT AND PARTITION PLOTS.

Genus	Species	Code for Biplots	Code for clade level	Code used at the genus level
Galerida	*magnirostris*	G.mag	A	A
Galerida	*deva*	G.dev	A	A
Galerida	*theklae*	G.the	A	A
Galerida	*malabarica*	G.mal	A	A
Galerida	*cristata*	G.cri	A	A
Galerida	*macrorhyncha*	G.mac	A	A
Calandrella	*cinerea*	C.cin	A	B
Calandrella	*acutirostris*	C.acu	A	B
Calandrella	*erlanger*	C.erl	A	B
Calandrella	*brachydactyla*	C.bra	A	B
Eremophila	*bilopha*	E.bil	A	C
Eremophila	*alpestris*	E.alp	A	C
Alauda	*leucotis*	A.leu	A	D
Chersophilus	*duponti*	Ch.dup	A	E
Alaudala	*cheleensis*	Al.che	A	F
Alaudala	*rufescens*	Al.ruf	A	F
Alaudala	*somalica*	Al.som	A	F
Alaudala	*raytal*	Al.ray	A	F
Melanocorypha	*maxima*	M.max	A	G
Melanocorypha	*bimaculata*	M.bim	A	G
Melanocorypha	*yeltoniensis*	M.yel	A	G
Melanocorypha	*calandra*	M.cal	A	G
Spizocorys	*conirostris*	S.con	A	H
Spizocorys	*fringillaris*	S.fri	A	H
Spizocorys	*sclateri*	S.scl	A	H
Spizocorys	*starki*	S.sta	A	H
Lullula	*arborea*	L.arb	A	I
Mirafra	*africana*	MiS.afr	B	J
Mirafra	*angolensis*	MiS.ang	B	J
Mirafra	*cheniana*	MiS.che	B	J
Mirafra	*gilletti*	MiS.gil	B	J
Mirafra	*pulpa*	MiS.pul	B	J
Mirafra	*hypermetra*	MiS.hyp	B	J

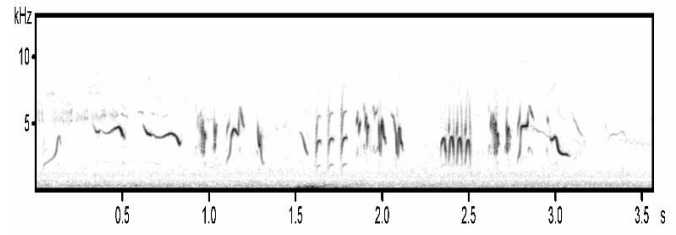
Mirafra	*passerina*	MiS.pas	B	J
Mirafra	*rufocinnamomea*	MiS.ruf	B	J
Mirafra	*fasciolata*	MiS.fas	B	J
Mirafra	*apiata*	MiS.api	B	J
Mirafra	*javanica*	MiN.jav	B	K
Mirafra	*microptera*	MiN.mic	B	K
Mirafra	*assamica*	MiN.ass	B	K
Mirafra	*erythrocephala*	MiN.erc	B	K
Mirafra	*affinis*	MiN.aff	B	K
Mirafra	*cantillans*	MiN.can	B	K
Mirafra	*erythroptera*	MiN.ert	B	K
Heteromirafra	*ruddi*	H.rud	B	L
Calendulauda	*barlowi*	Ca.bar	B	M
Calendulauda	*erythrochlamys*	Ca.ery	B	M
Calendulauda	*albescens*	Ca.alb	B	M
Calendulauda	*burra*	Ca.bur	B	M
Calendulauda	*africanoides*	Ca.afr	B	M
Calendulauda	*alopex*	Ca.alo	B	M
Calendulauda	*sabota*	Ca.sab	B	M
Certhilauda	*brevirostris*	Ce.bre	C	N
Certhilauda	*curvirostris*	Ce.cur	C	N
Certhilauda	*semitorquata*	Ce.sem	C	N
Certhilauda	*benguelensis*	Ce.ben	C	N
Certhilauda	*subcoronata*	Ce.sub	C	N
Certhilauda	*chuana*	Ce.chu	C	N
Eremopterix	*nigriceps*	Er.nig	C	O
Eremopterix	*griseus*	Er.gri	C	O
Eremopterix	*signatus*	Er.sig	C	O
Eremopterix	*leucotis*	Er.leu	C	O
Eremopterix	*verticalis*	Er.ver	C	O
Ammomanes	*cintura*	Am.cin	C	P
Ammomanes	*deserti*	Am.des	C	P
Ramphocorys	*clotbey*	R.clo	C	Q
Ammomanopsis	*grayi*	Amm.gra	C	R
Chersomanes	*albofasciata*	Che.alb	C	S
Alaemon	*alaudipes*	Ala.ala	C	T

APPENDIX 3.2. MULTIPLE SPECTROGRAMS OF SONG STROPHES GENERATED FROM DIFFERENT INDIVIDUALS REPRESENTING DIFFERENT SPECIES. **ML** - MACAULAY LIBRARY OF NATURAL SOUNDS, **XC** - XENO-CANTO, **PA** - PER ALSTRÖM, **DS** - DAWIE DE SWARDT, **DA** - DESMOND ALLEN, **AV**- AVOCET). FOR FIGURE NUMBERING, E.G. (**3.2.1 A-F**), **3** DENOTES CHAPTER NUMBER, **2** DENOTES APPENDIX NUMBER, **1** DENOTES GENUS, **A-F** DENOTE SPECIES.

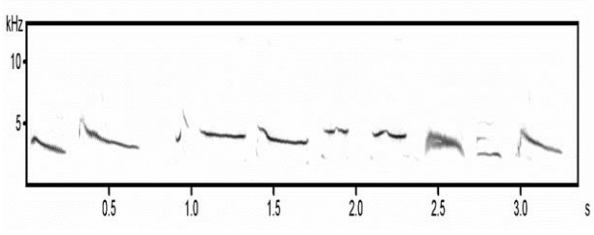
Galerida

Appendix 3.2.1 a-f

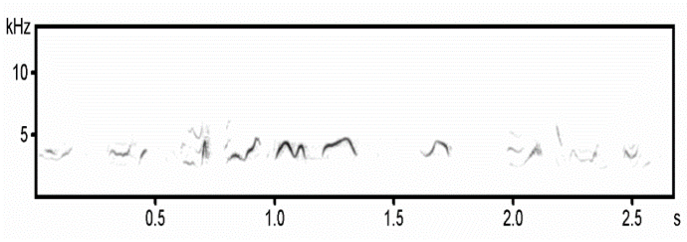
G. cristata



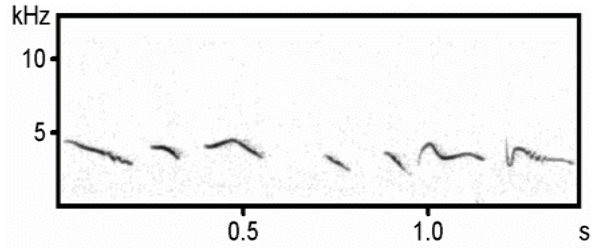
a. ML86284



PA_91_02_303

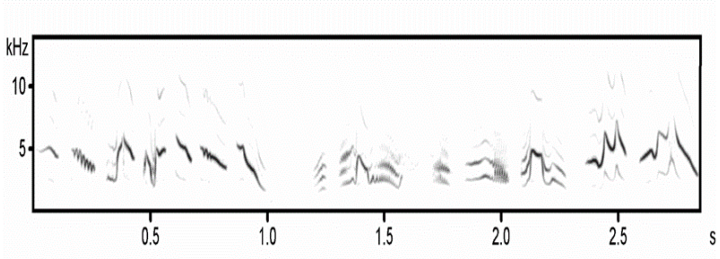


XC361968

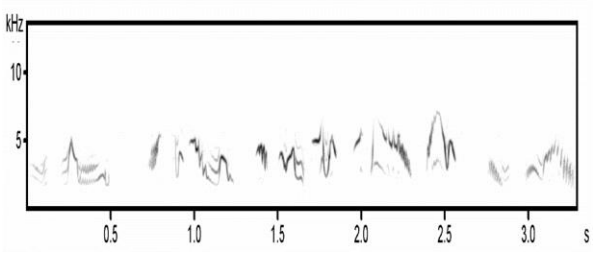


XC91636

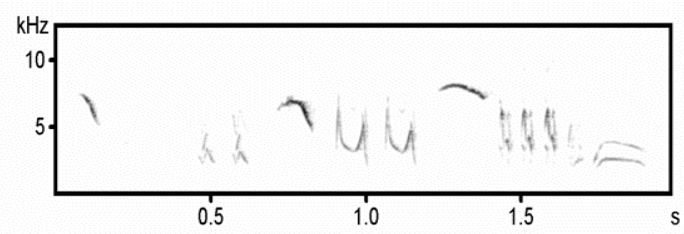
G. theklae



b. XC267275

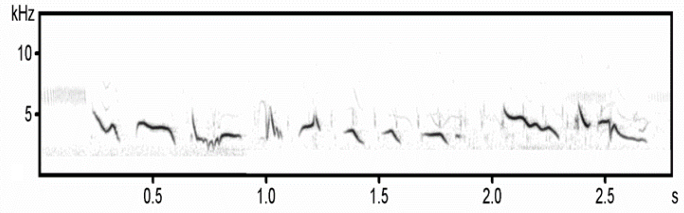


XC300306

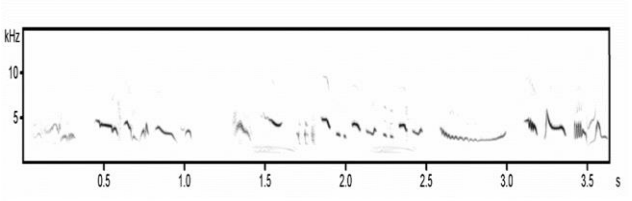


XC370396

G. malabarica

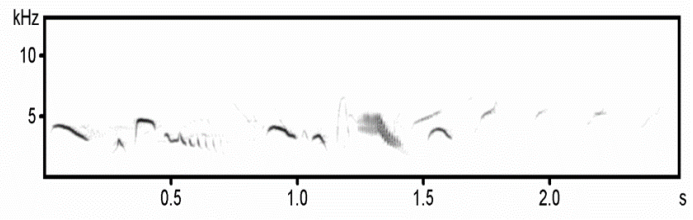


c. XC44811

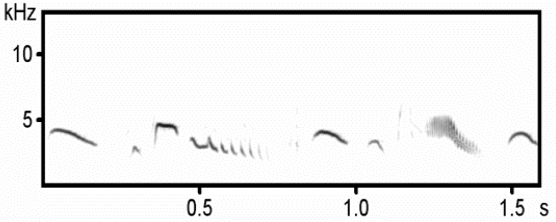


PA_93_01_48-51

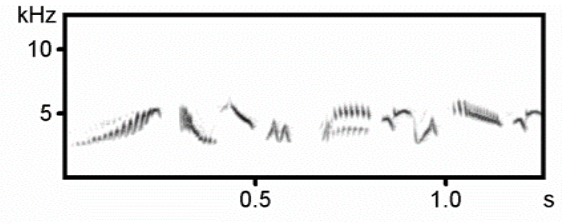
G. deva



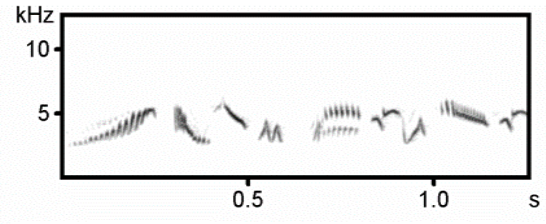
d. XC369214



XC369216

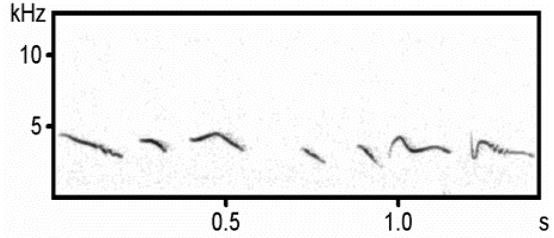


XC369215

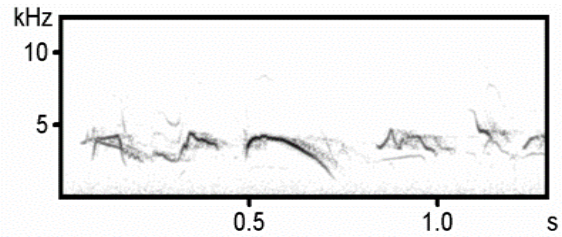


XC369217

G. macrorhyncha

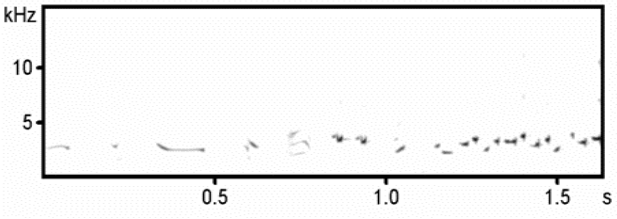


e. XC91636

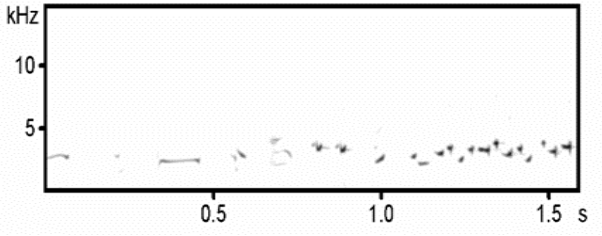


XC317662

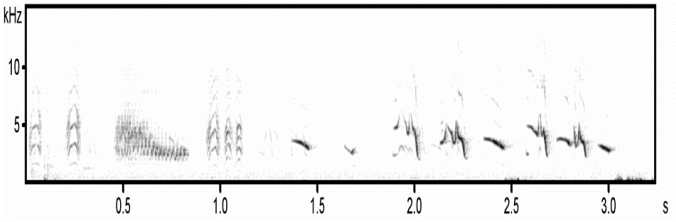
G. magnirostris



f. PA_1002



PA_1038



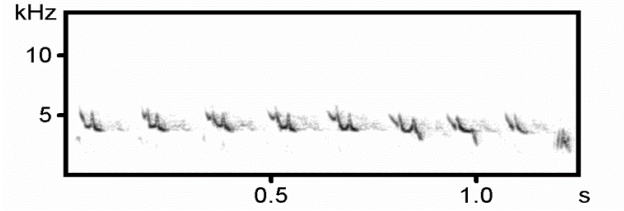
XC313733

XC280313

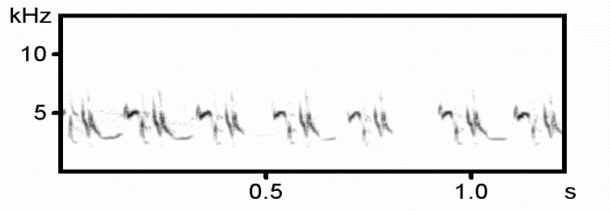
Calandrella

Appendix 3.2.2 a-d

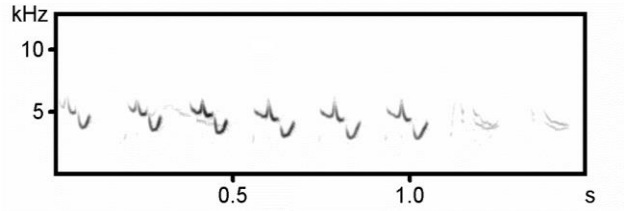
C. cinerea



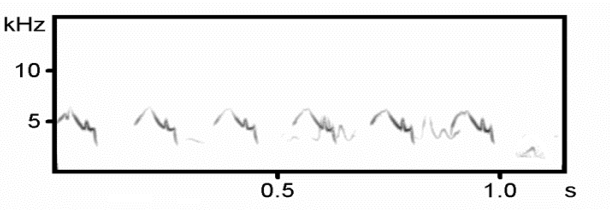
a. XC279914



XC279922

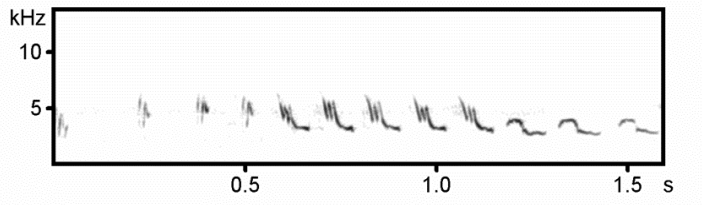


XC279923

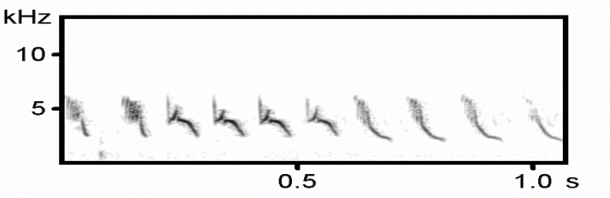


PA_2-1092

C. brachydactyla

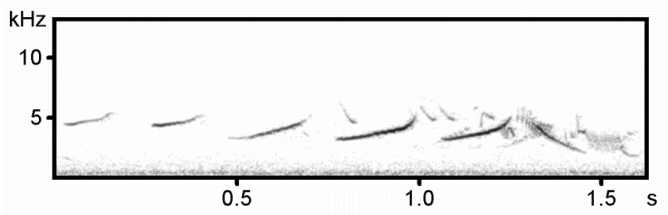


b. XC295988



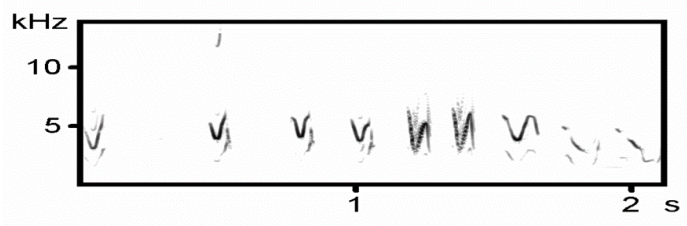
XC164215

C. acutirostris



c. XC176708

C. erlanger

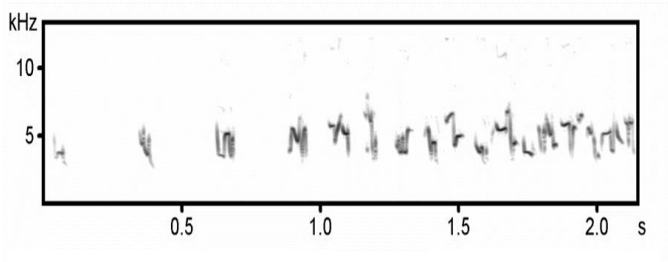


d. XC300037

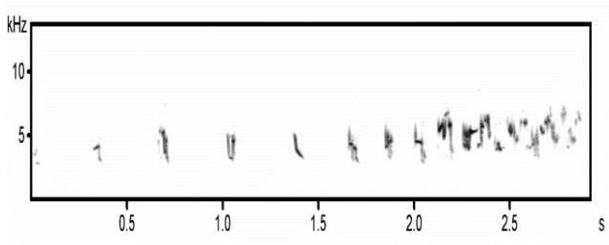
Eremophila

Appendix 3.2.3 a-b

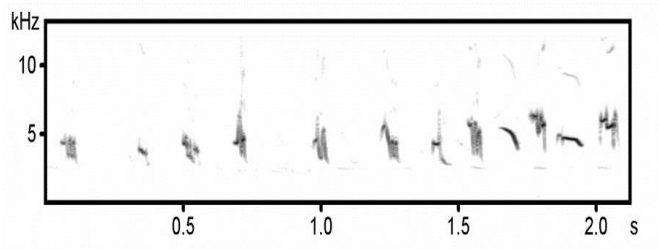
E. alpestris



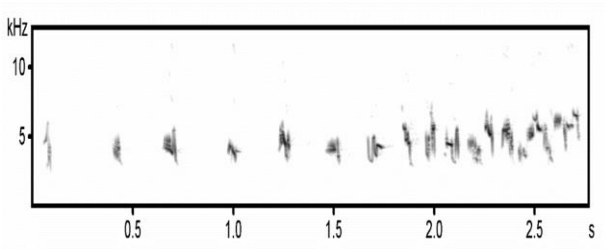
a. ML73842



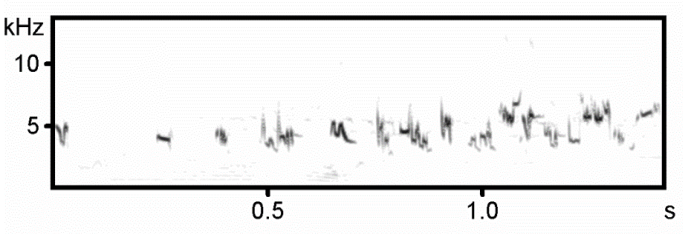
ML118662



ML516603

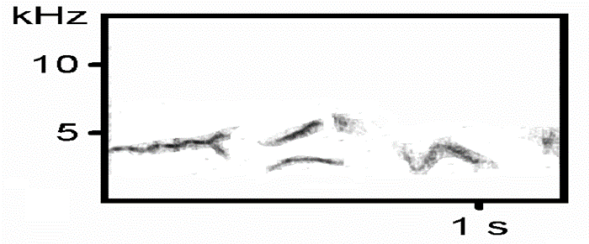


ML50257



XC293914

E. bilopha

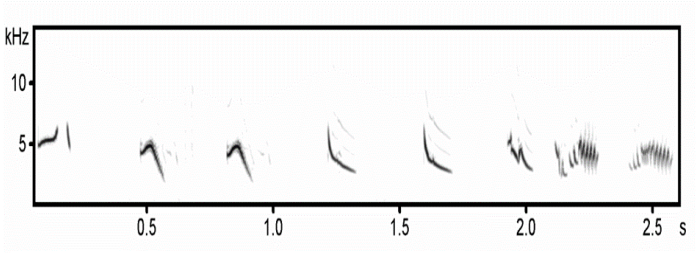


b. XC175385

Alauda

Appendix 3.2.4 a

A. leucoptera

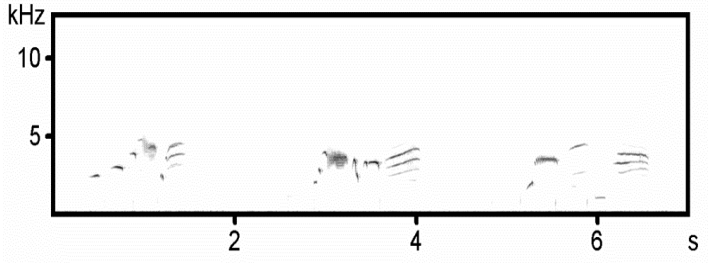


a. XC236707

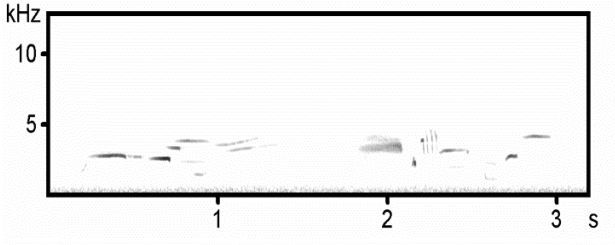
Chersophilus

Appendix 3.2.5 a

C. duponti



a. XC140665

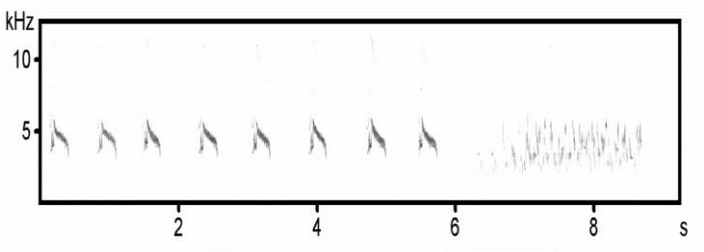


XC315238

Alaudala

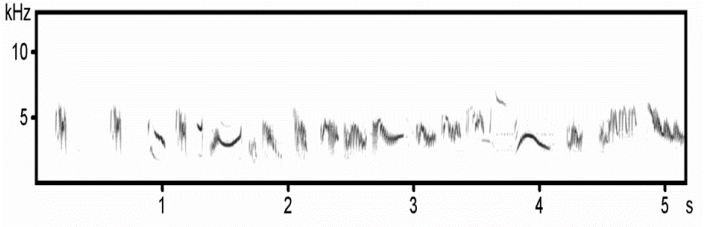
Appendix 3.2.6 a-d

A. rufescens

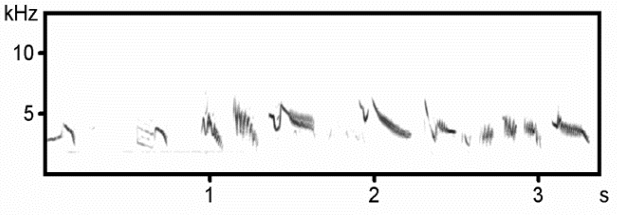


a. **XC175825**

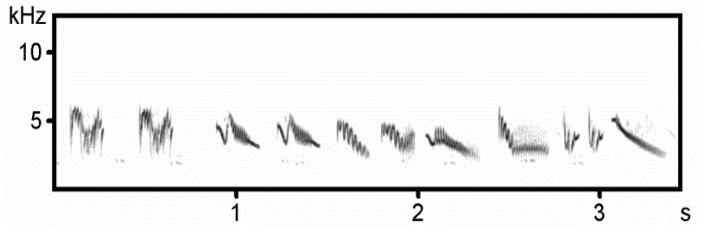
A. somalica



b. XC300030

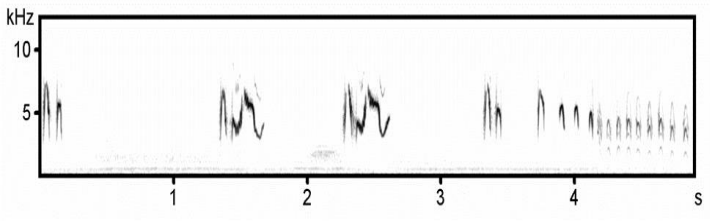


XC300031



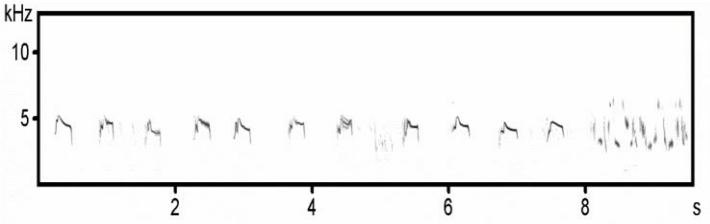
XC300033

A. raytal



c. PA_9104717

A. cheleensis

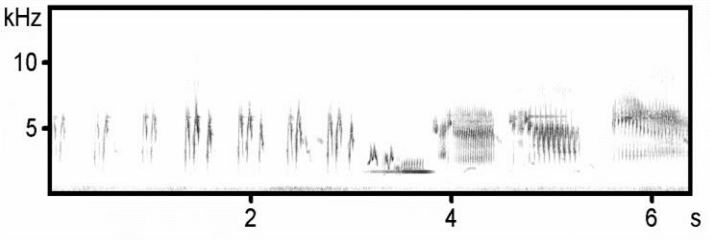


d. XC3162713

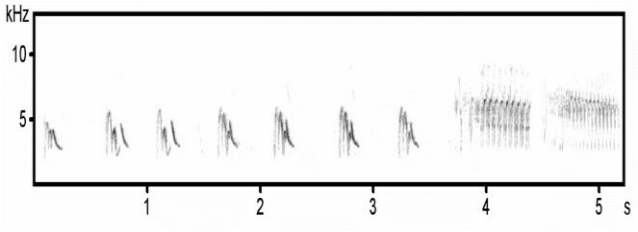
Melanocorypha

Appendix 3.2.7 a-d

M. calandra

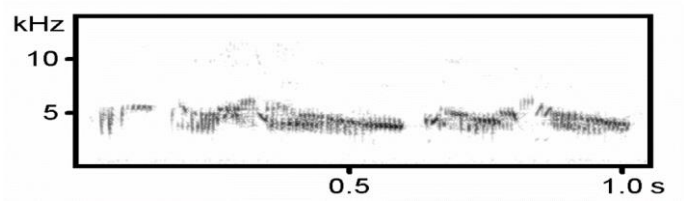


a. XC268744



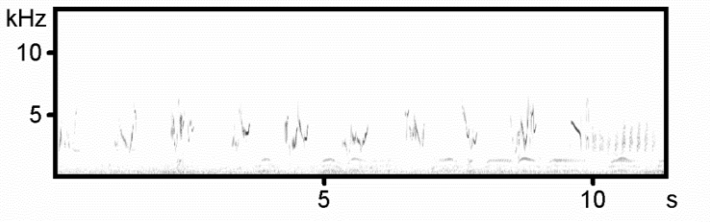
XC243479

M. bimaculata

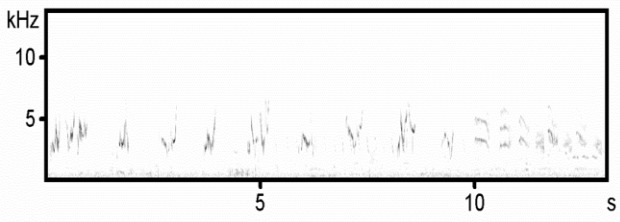


b. XC257185

M. maxima

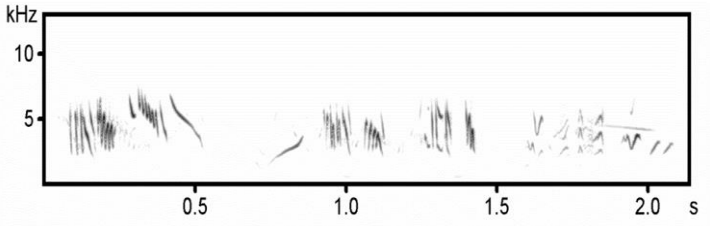


c. XC110990

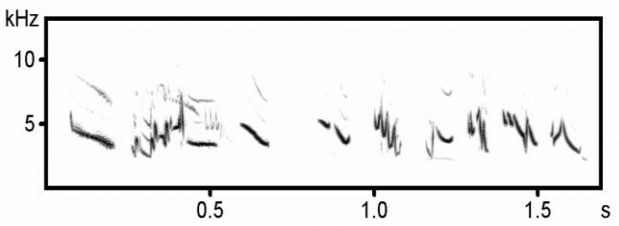


XC110991

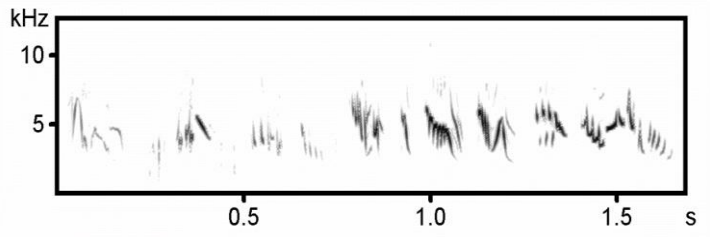
M. yeltoniensis



d. XC108873



XC154499

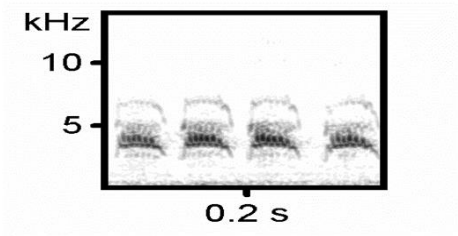


XC145076

Spizocorys

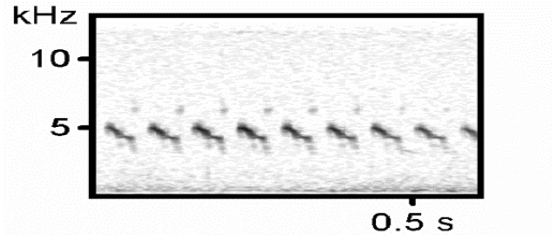
Appendix 3.2.8 a-d

S. conirostris



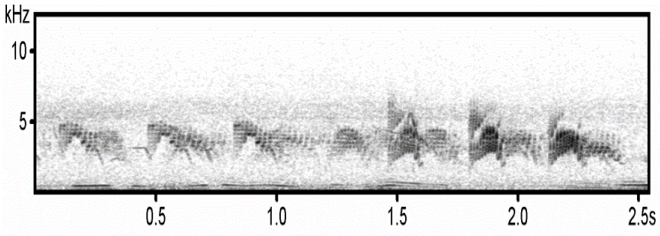
a. PA_1008

S. fringillaris



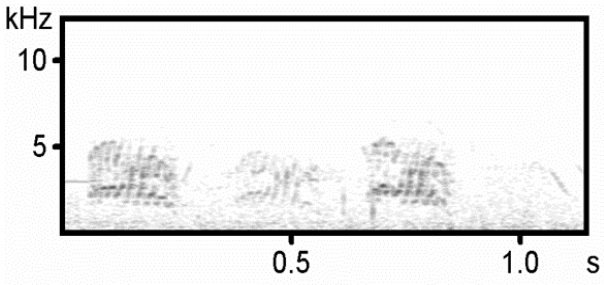
b. ML80706

S. starki



c. ML61156

S. sclateri

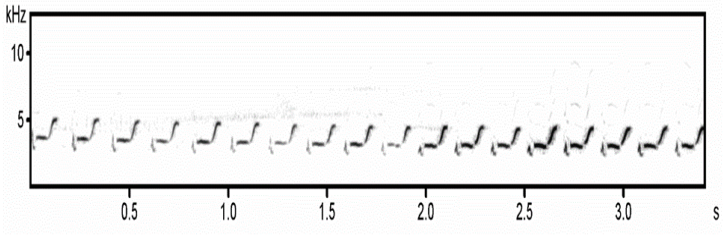


d. ML61157

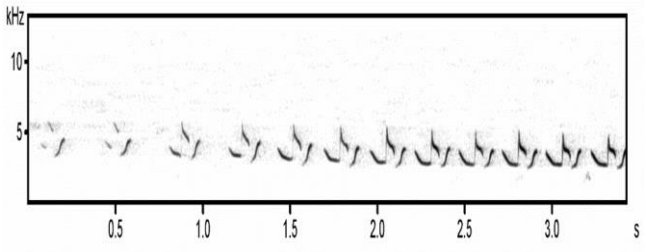
Lullula

Appendix 3.2.9 a

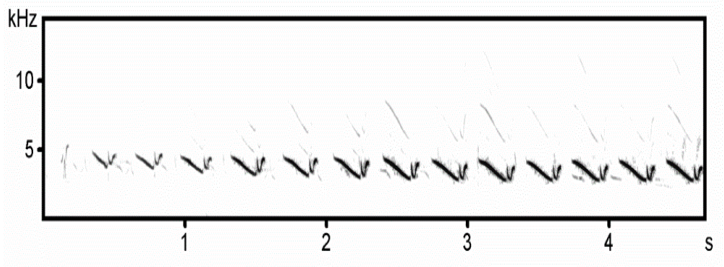
L. arborea



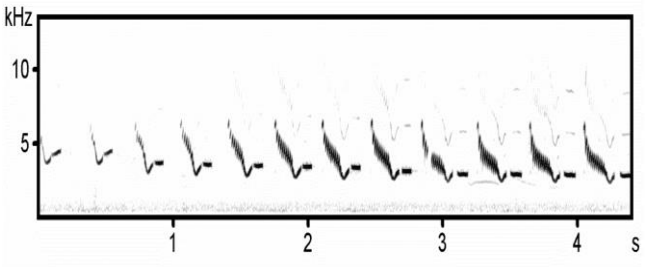
a. XC374316



XC373126



XC374310

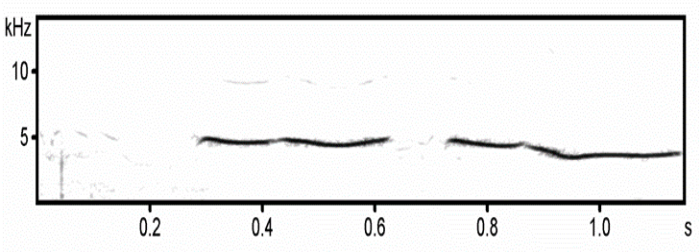


XC311724

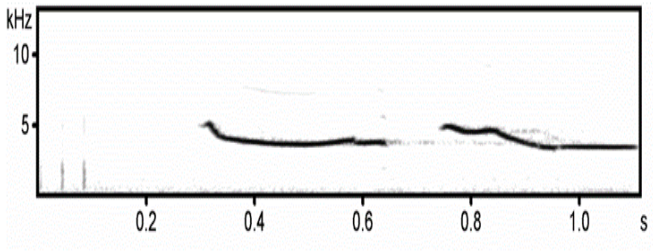
Mirafra

Appendix 3.3.1 a-q

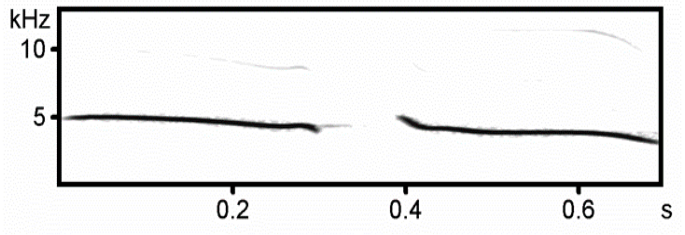
M. africana



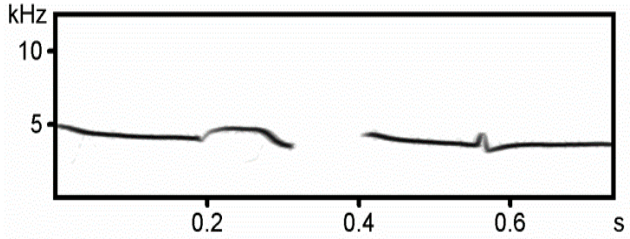
a. XC268628



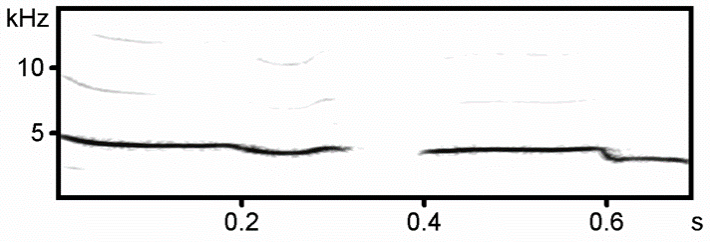
PA_1001



PA_1017

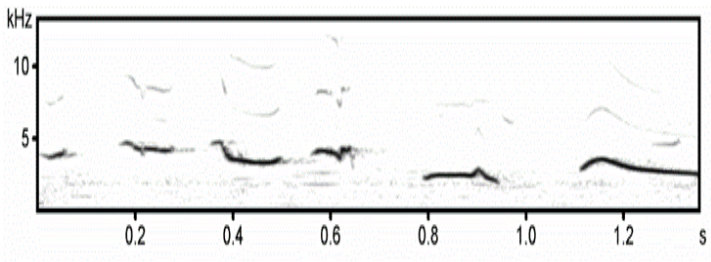


XC280455



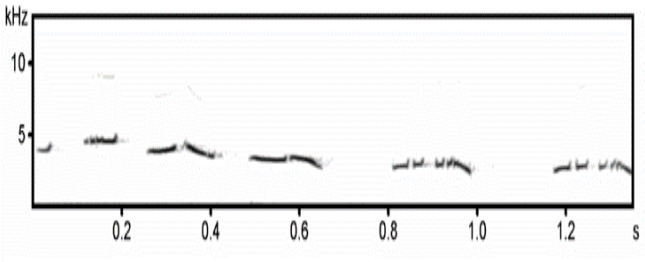
XC307246

M. hypermetra



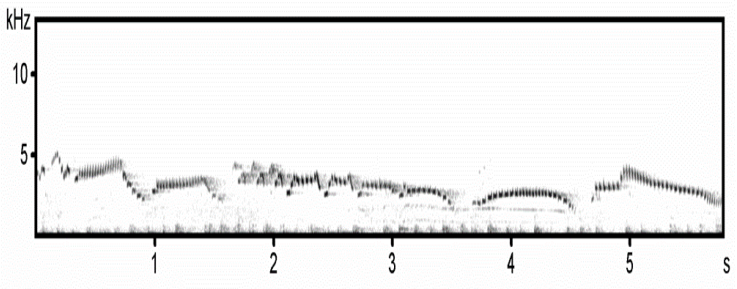
b. XC57941

b.

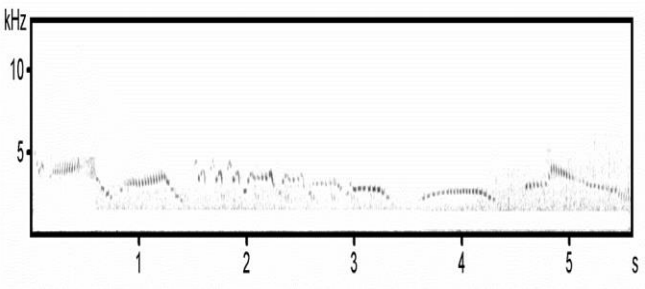


XC178954

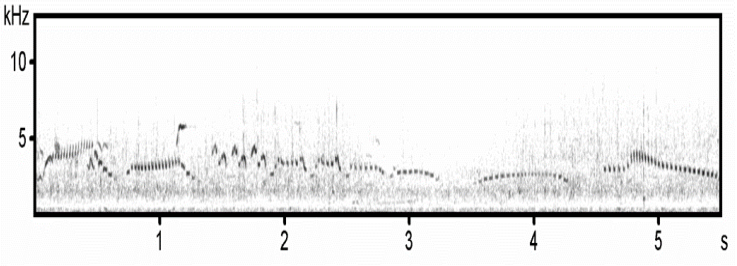
M. angolensis



c. ML101195

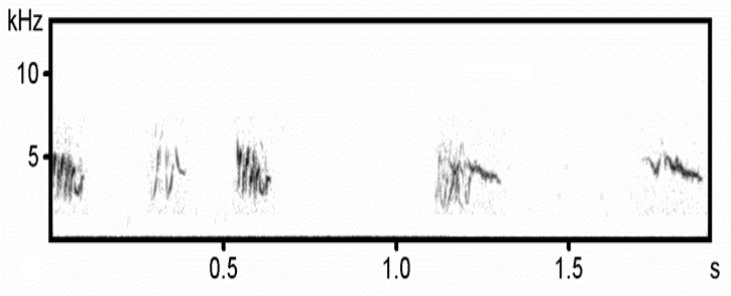


ML101192



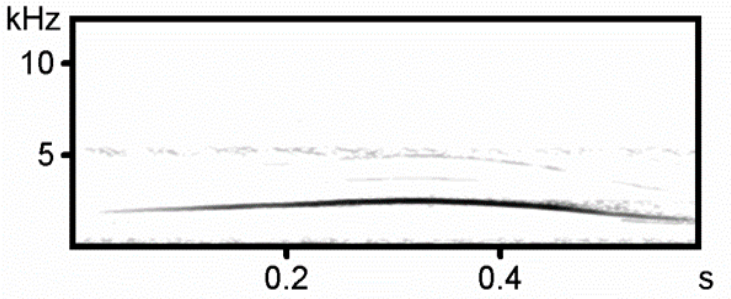
ML10193

M. gilletti



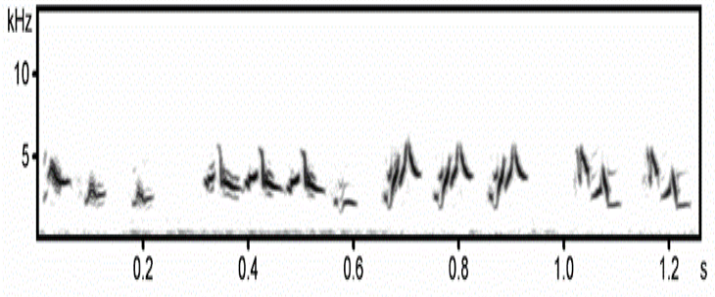
d. ML100216

M. pulpa

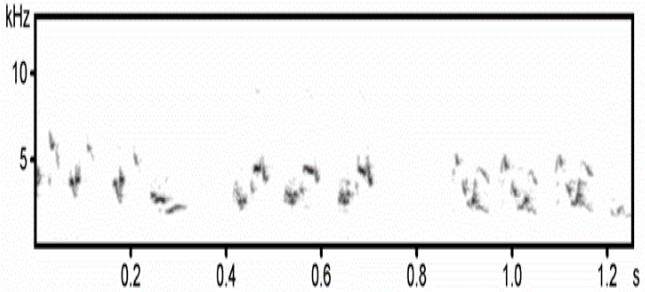


e. ML8044

M. cheniana

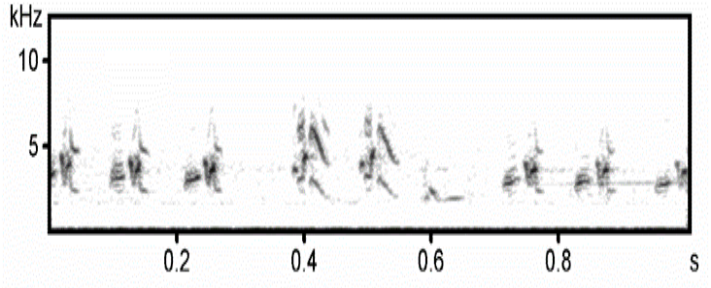


f. PA_1005

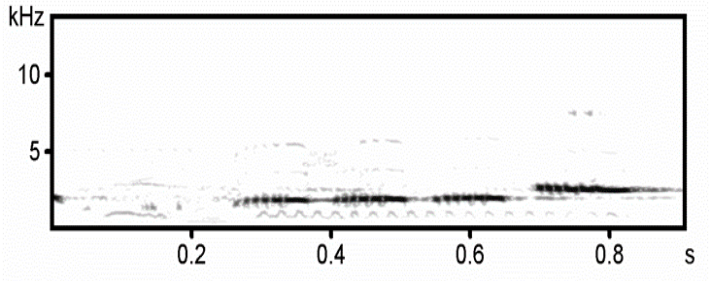


DS20160923

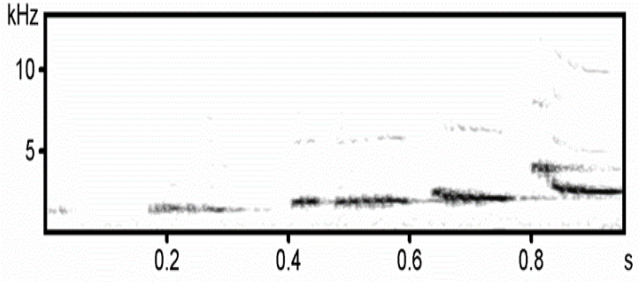
M. passerina



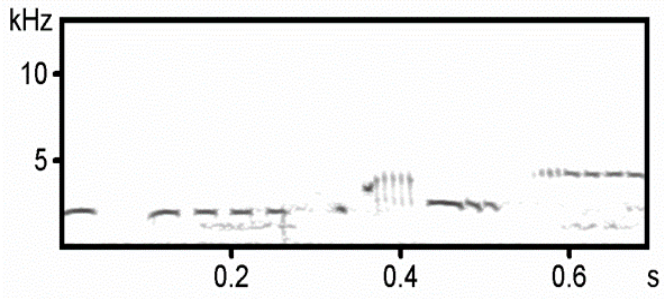
ML72592



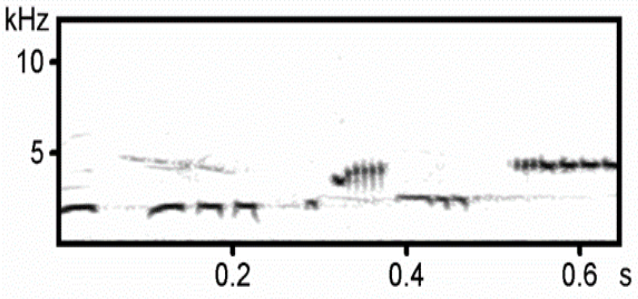
g. XC280457



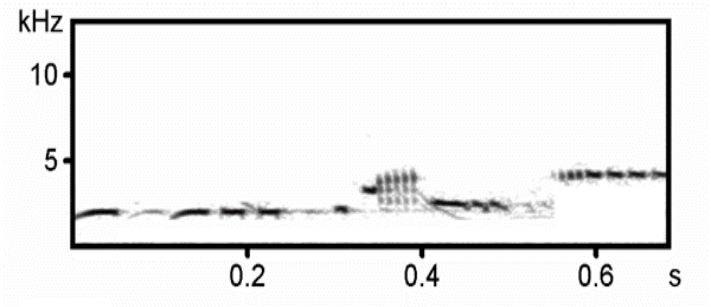
XC204673



ML61171

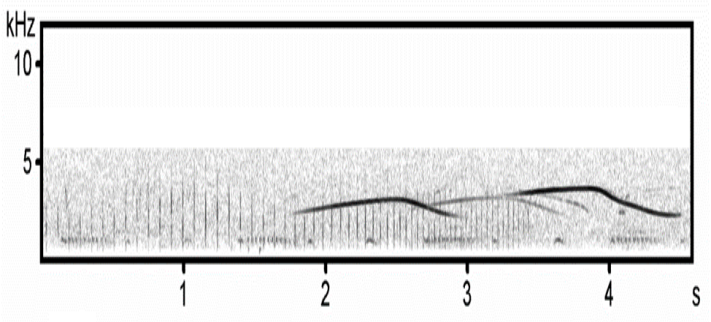


ML61155

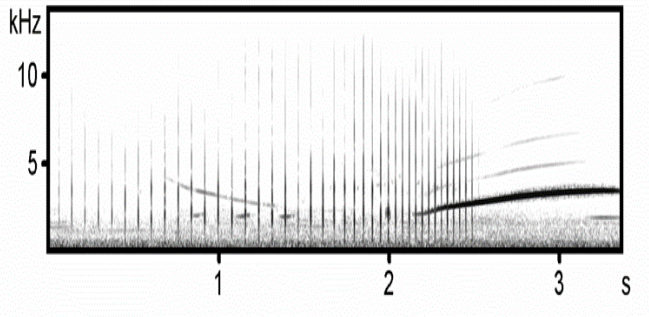


ML61146

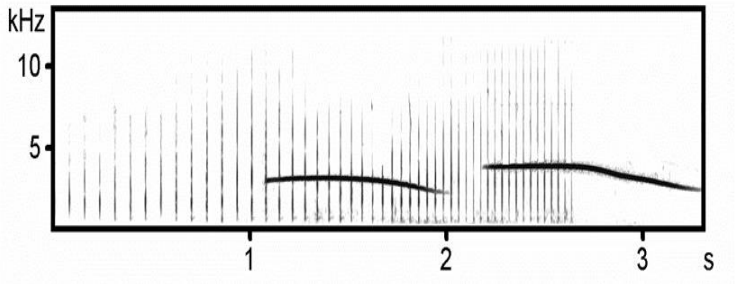
M. apiata



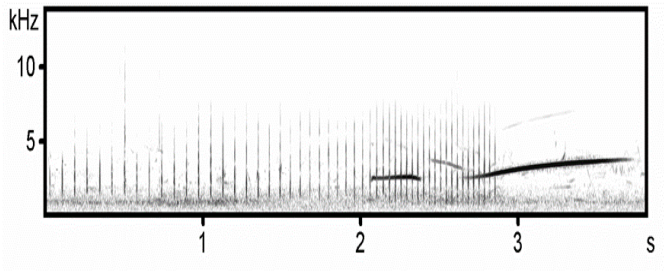
h. XC288867



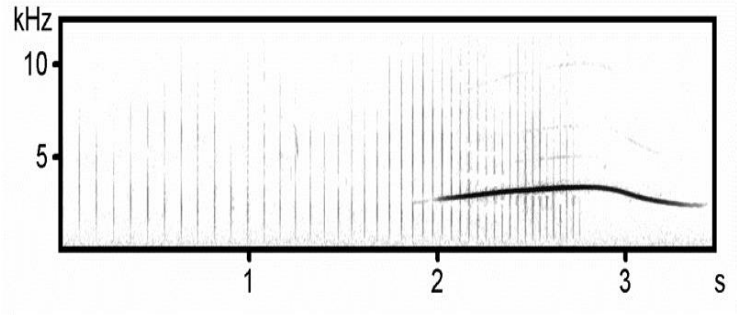
PA__1067



XC292640

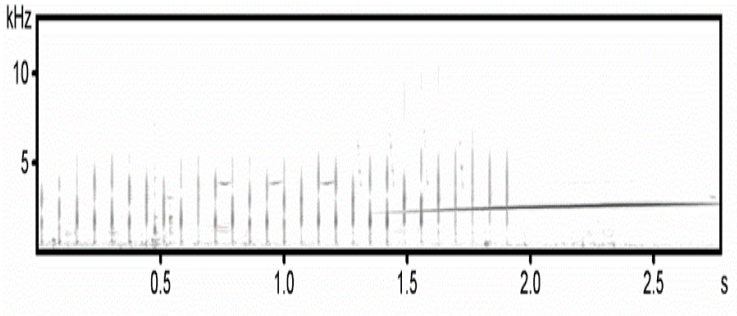


XC292650

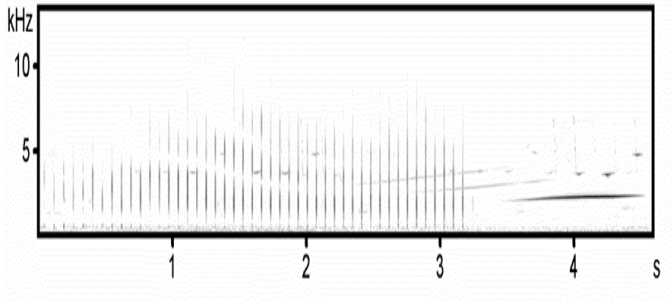


XC304509

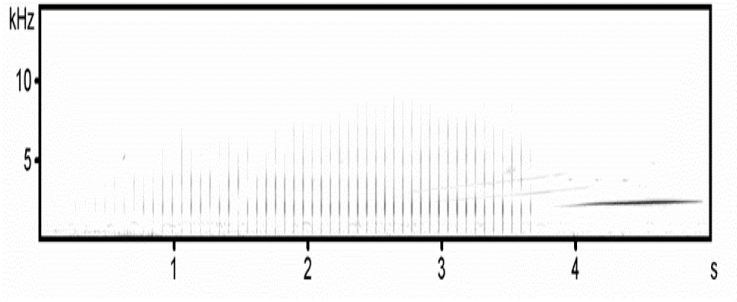
M. fasciolata



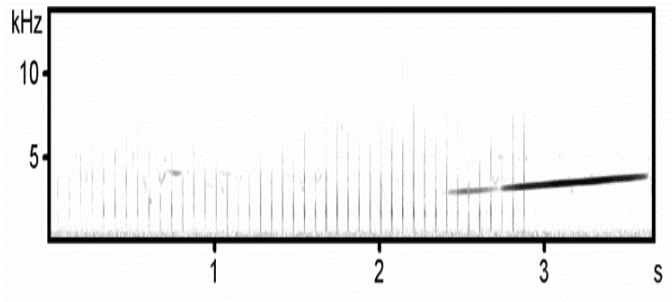
i. XC280466



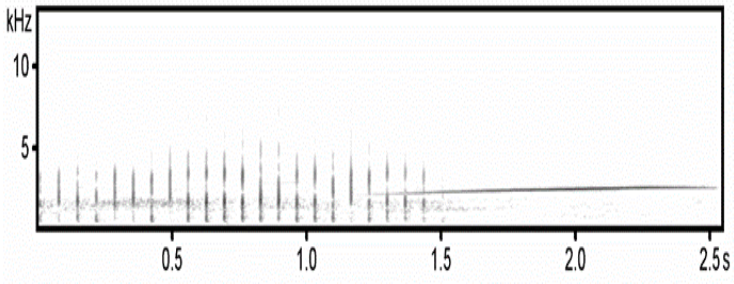
PA_2017-1114



PA__2017-1117

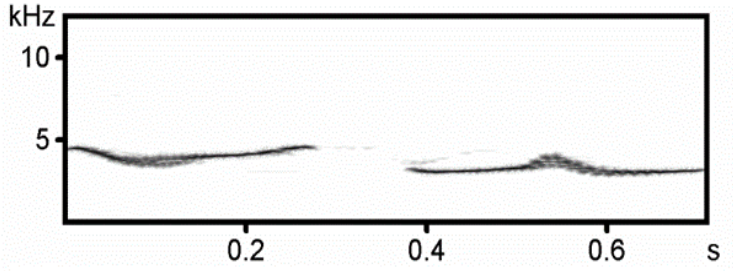


PA__2017-1125

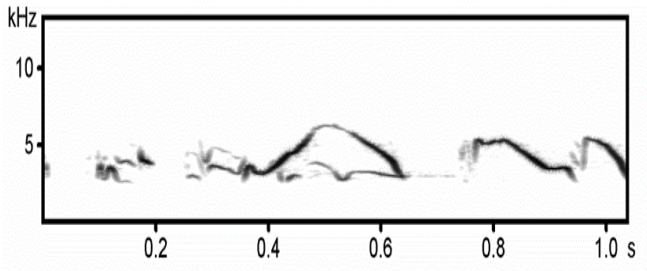


XC292634

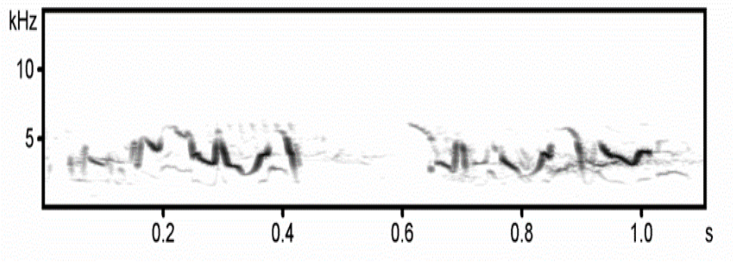
M. rufocinnamomea



j. XC270466

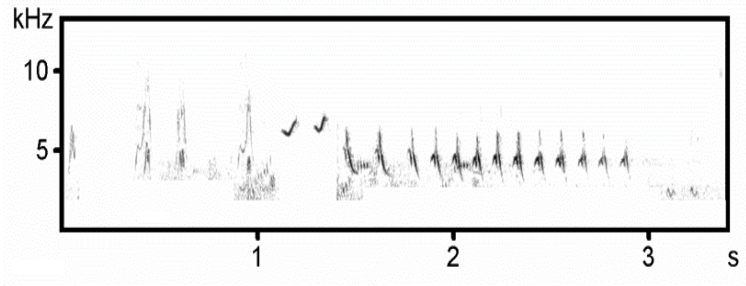


XC41316

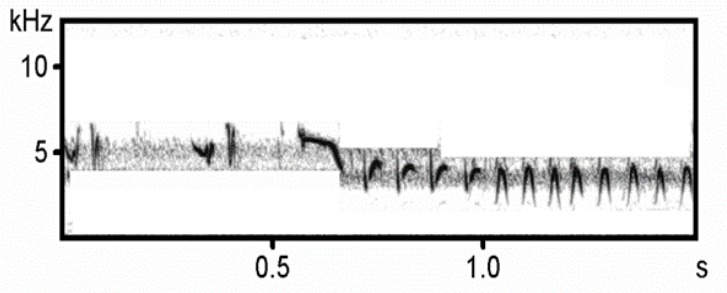


XC266874

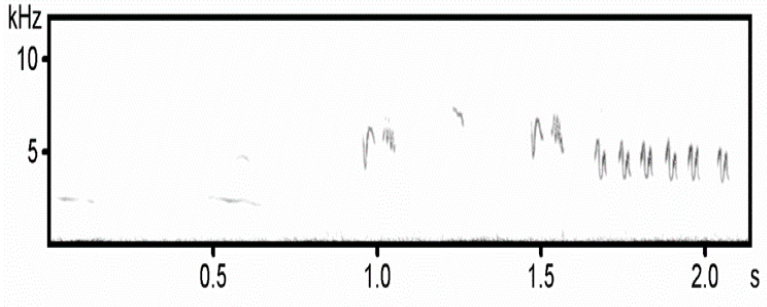
M. javanica



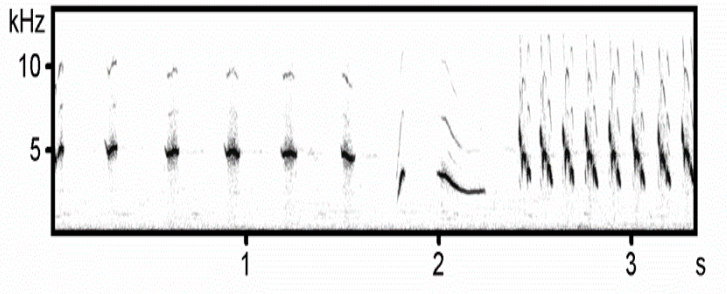
k. DA07-2-16



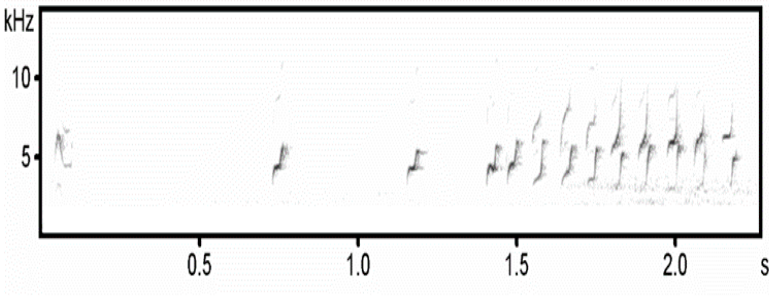
ML128310



ML128322

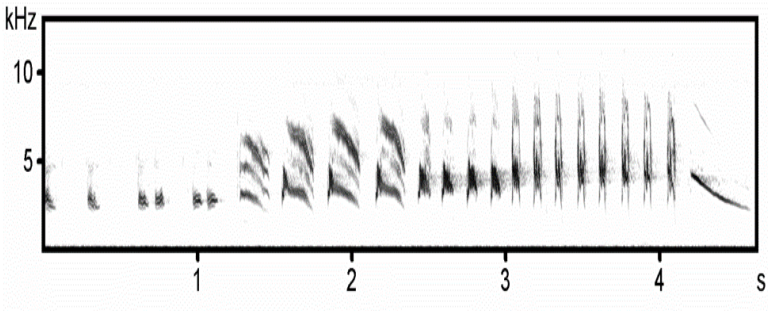


PA_87-679-720

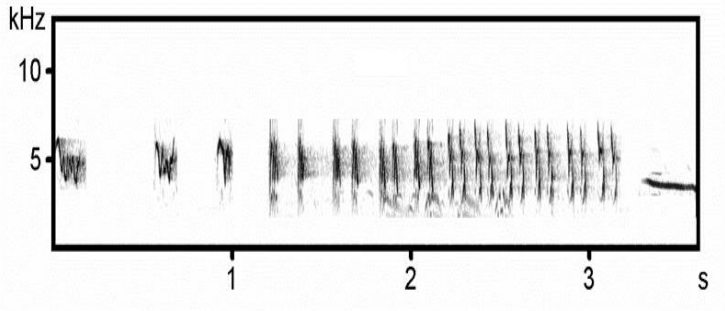


XC167898

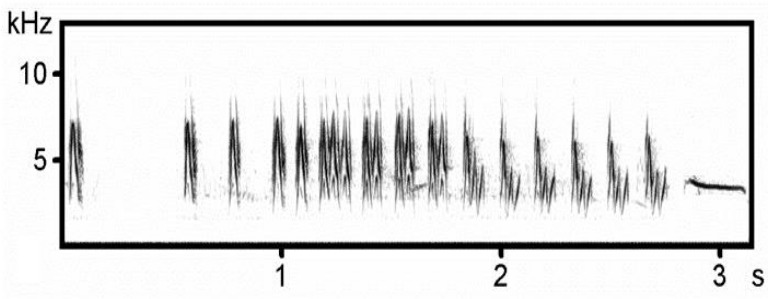
M. cantillans



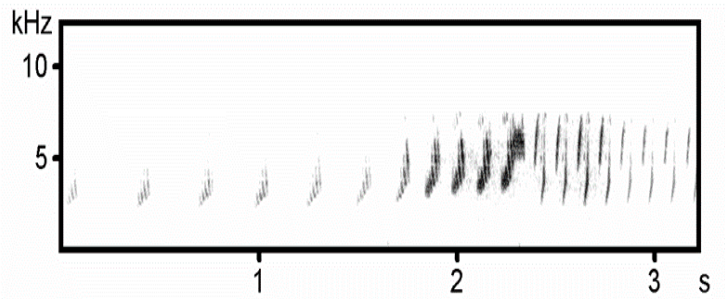
L. PA_98-01-40



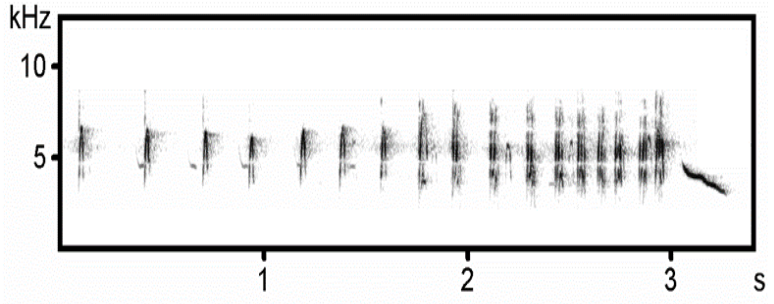
PA_98-01-39



PA_98-01-38

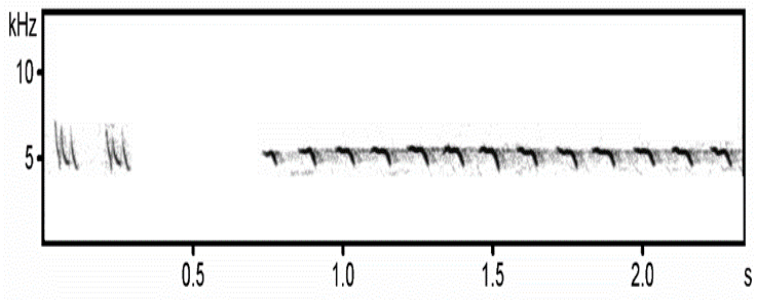


XC267260

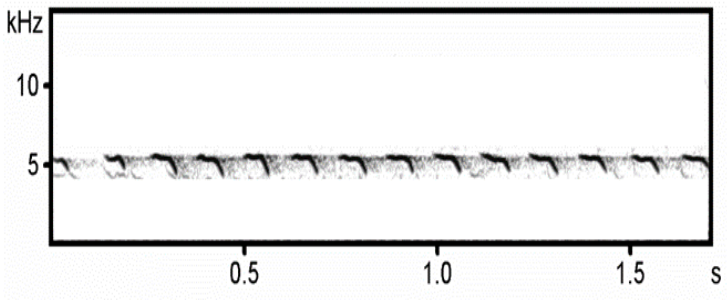


XC114273

M. microptera

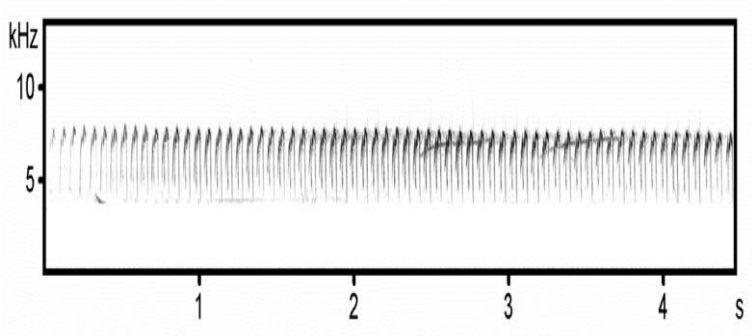


m. XC80381

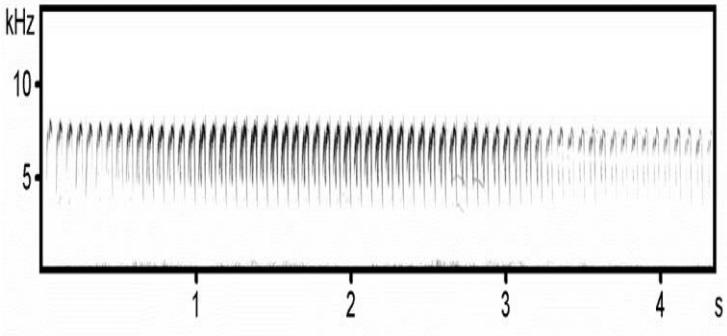


XC80380

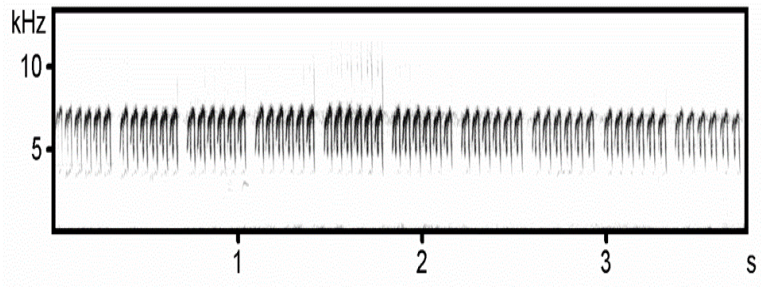
M. affinis



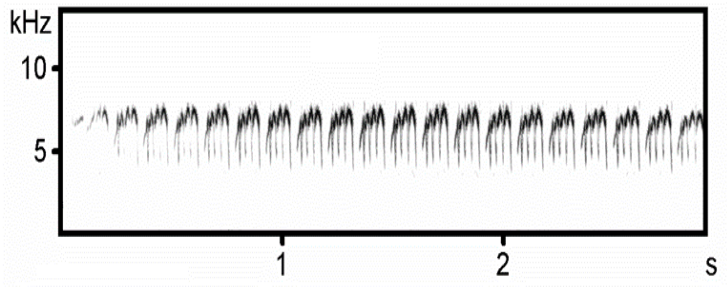
n. PA_93-01-18-21



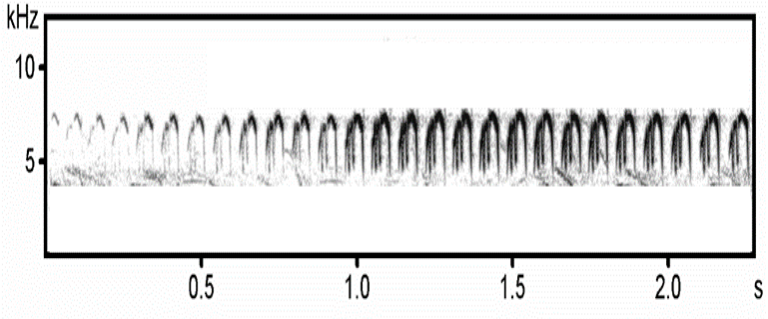
PA_93-01-23-28



PA_93-01-30

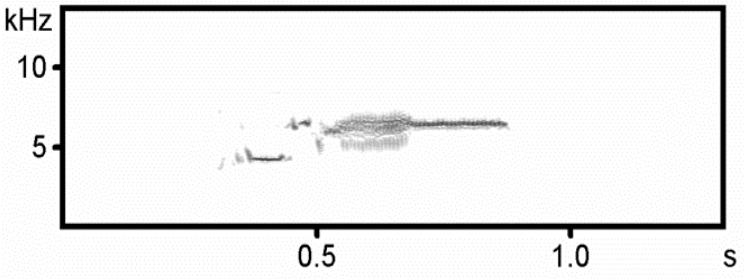


PA_93-01-23

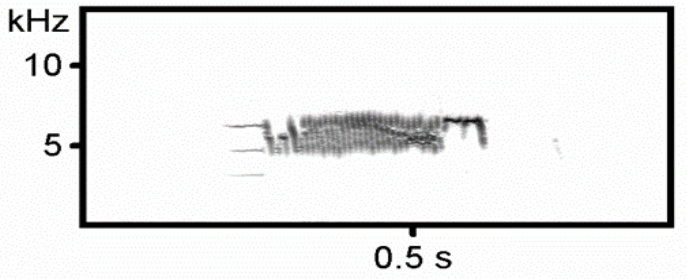


XC190819

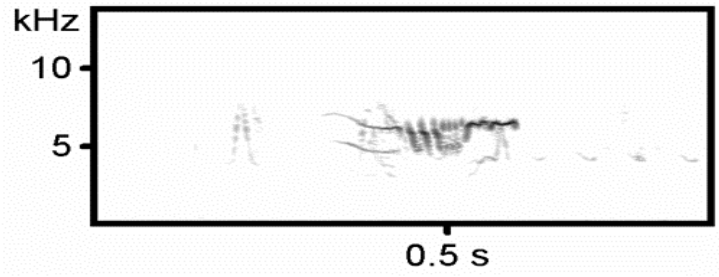
M. assamica



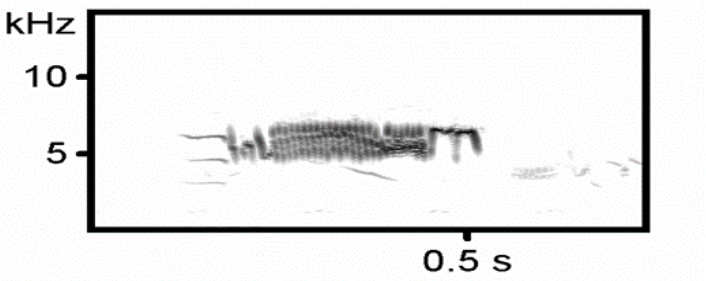
o. PA_94-01-60-61



PA_94-02-14

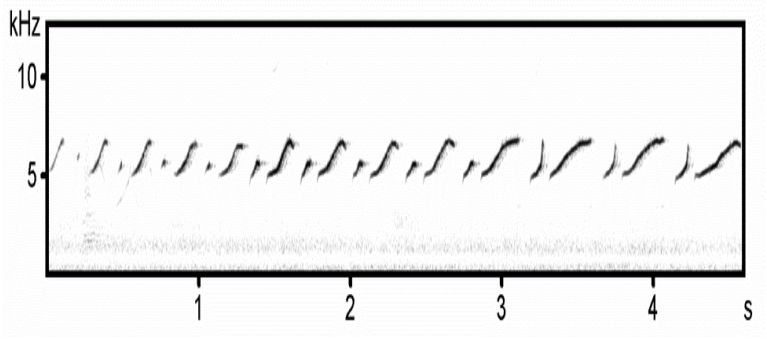


PA_94-02-02

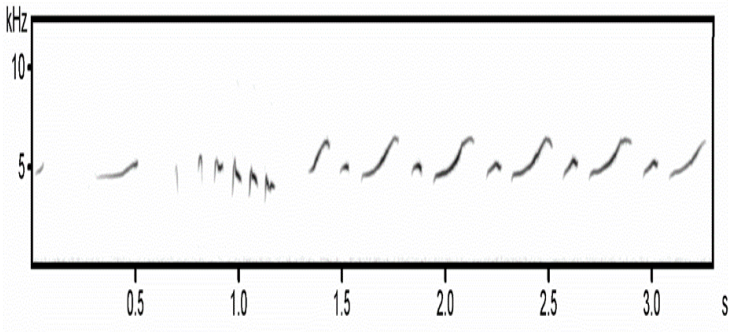


PA_94-02-22-28

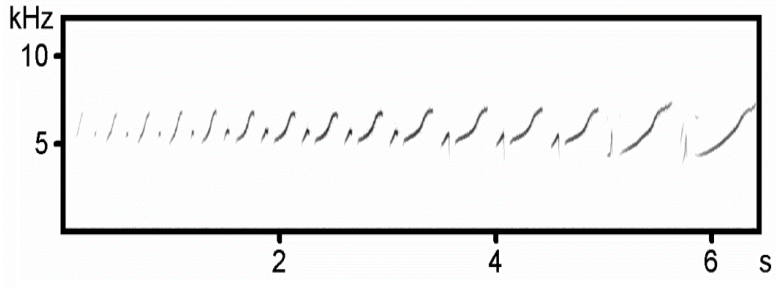
M. erythroptera



p. PA_97-04-40

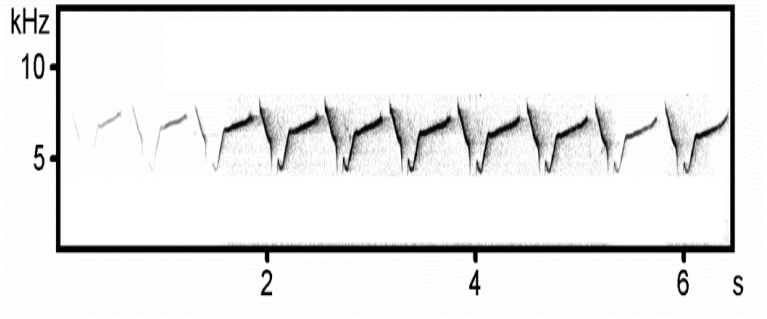


PA_97_04_39

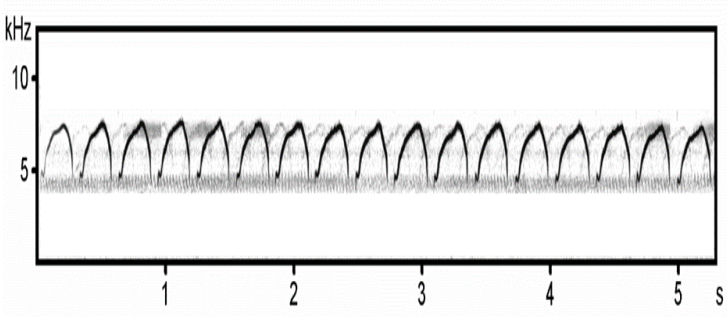


PA_91_01_006

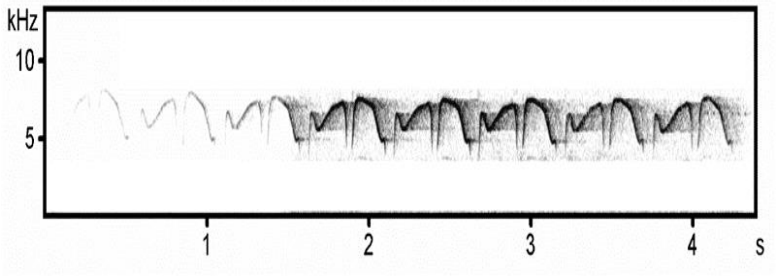
M. erythrocephala



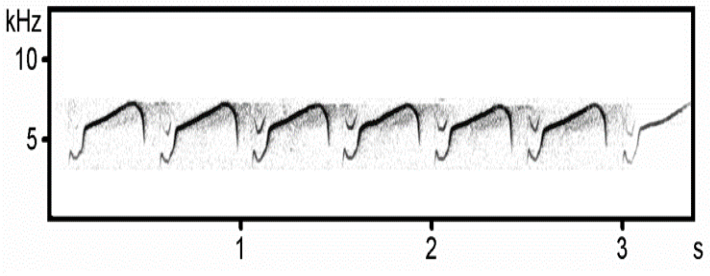
q. XC88228



XC124364



XC88226

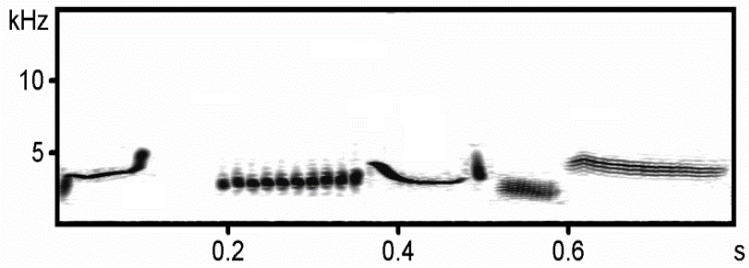


XC88229

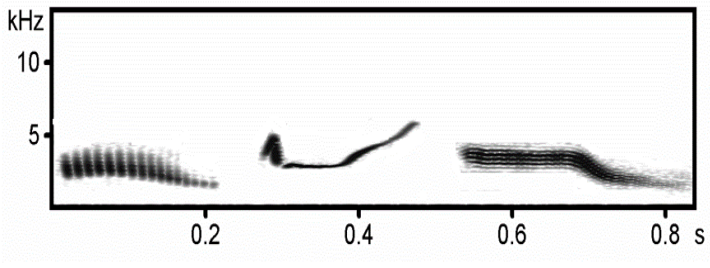
Heteromirafra

Appendix 3.3.2 a

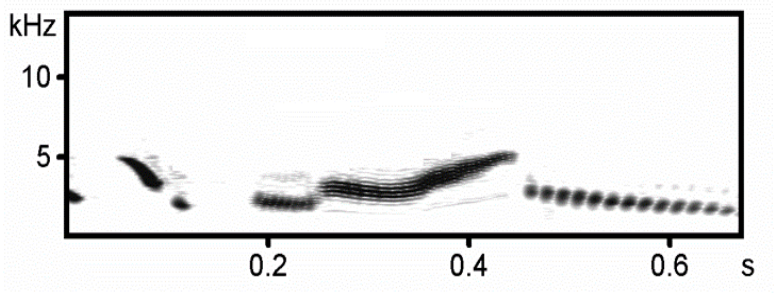
H. ruddi



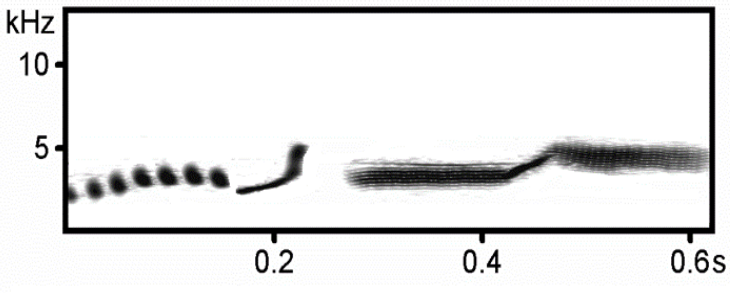
a. PA_2017-1056



PA_2017-1061



PA_2017-1099

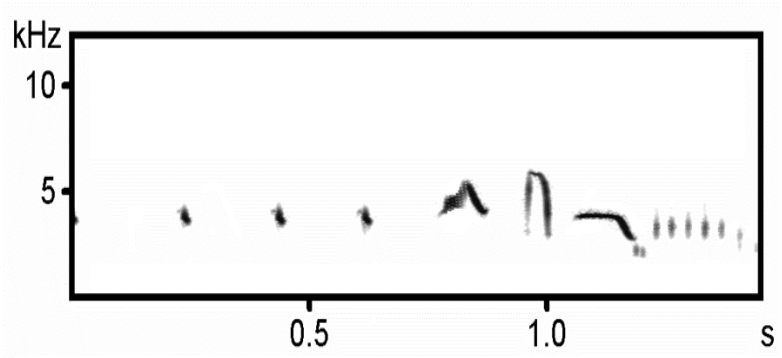


PA_2017-1105

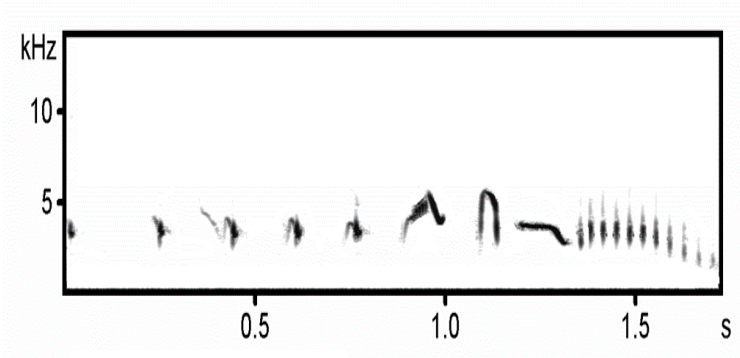
Calendulauda

Appendix 3.3.3 a-g

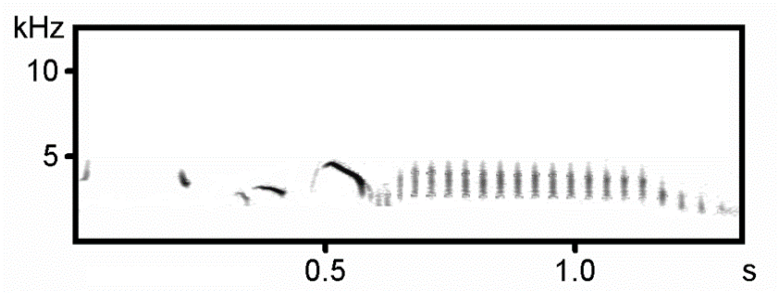
C. barlowi



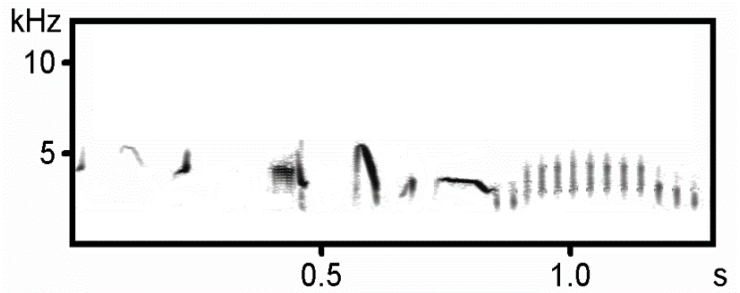
a. XC158041



PA_1010

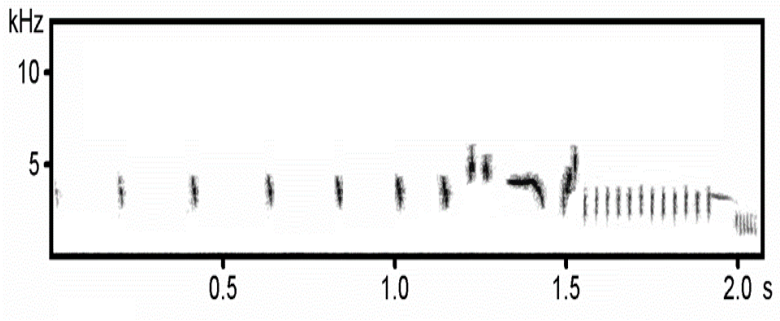


XC146425

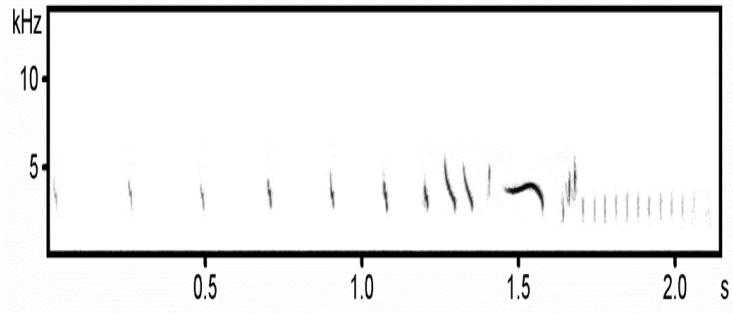


XC146426

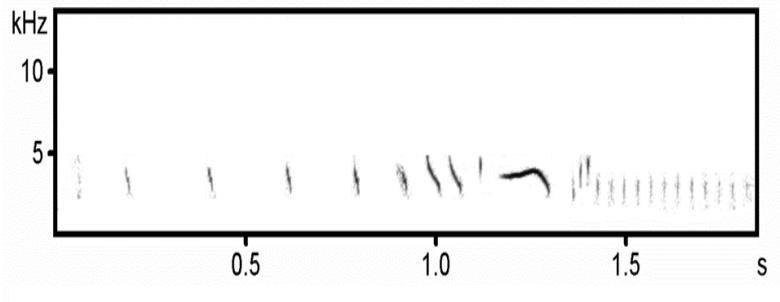
C. erythrochlamys



b. XC58517

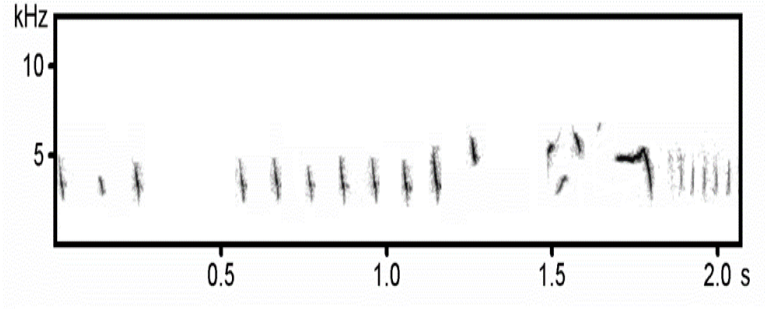


AV_08-0-22-1

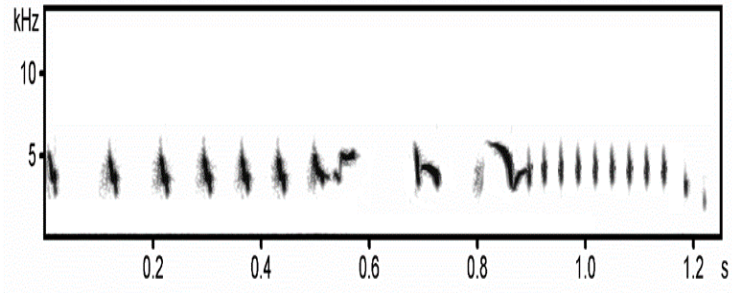


AV_08-0-22-2

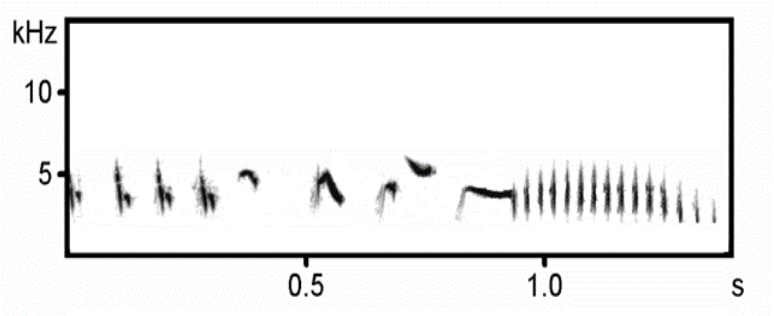
C. albescens



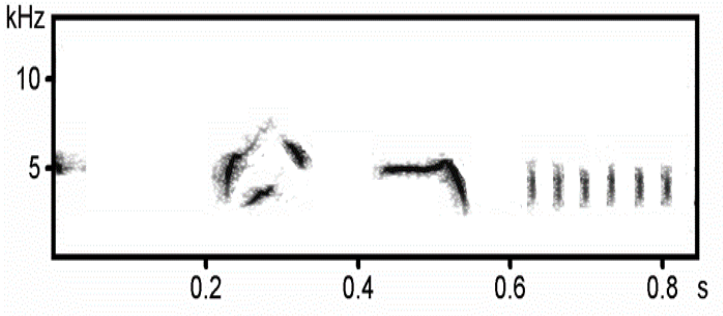
c. XC58017



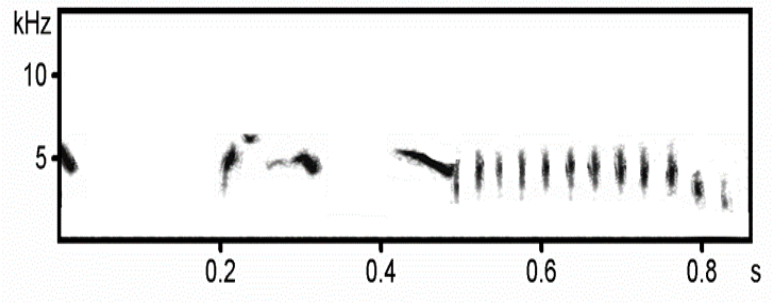
PA_1051



PA_1045

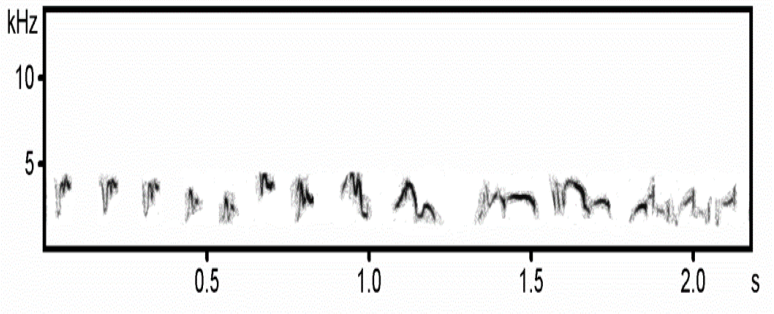


XC58018

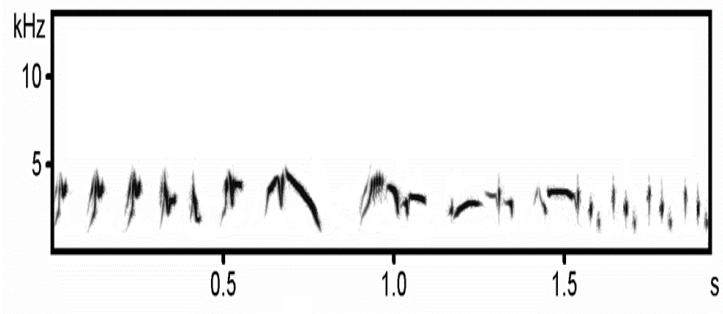


PA_1040

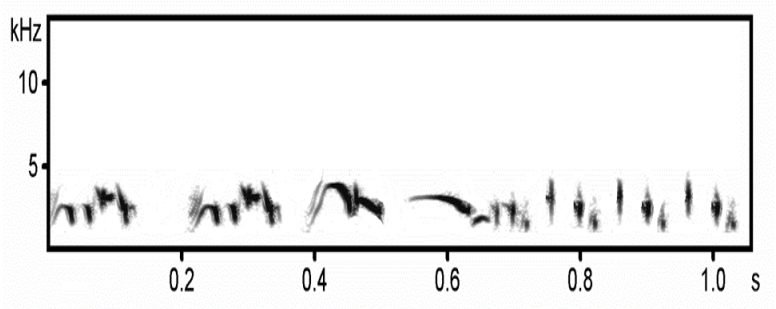
C. burra



d. XC146481

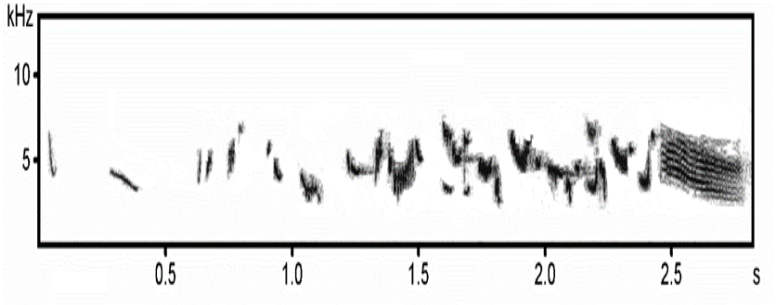


PA_1009

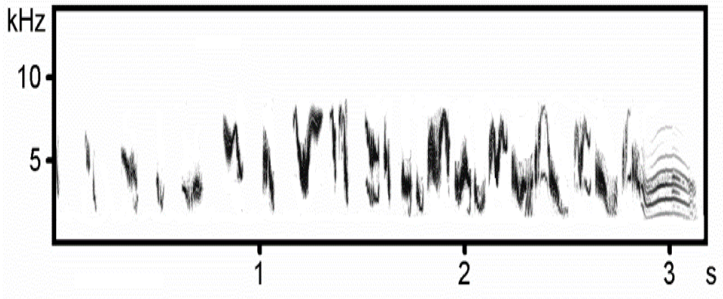


PA_1035

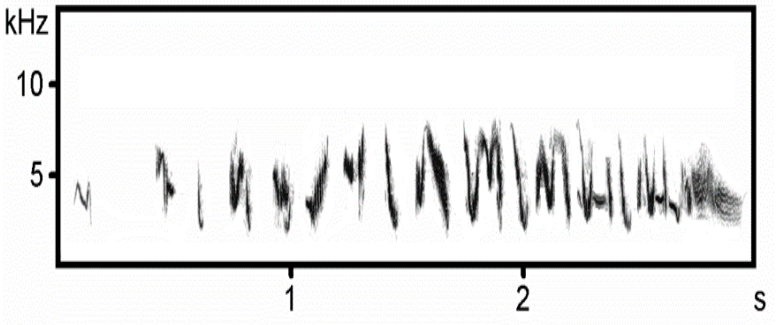
C. africanoides



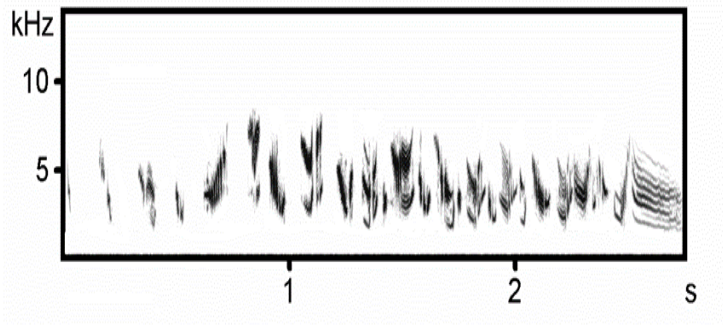
e. XC58708



PA_1021-1

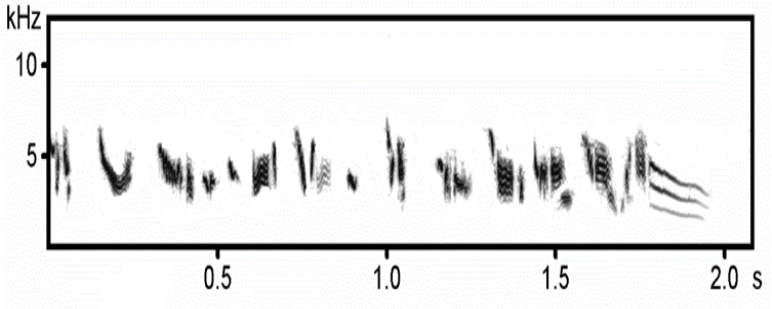


PA_1020

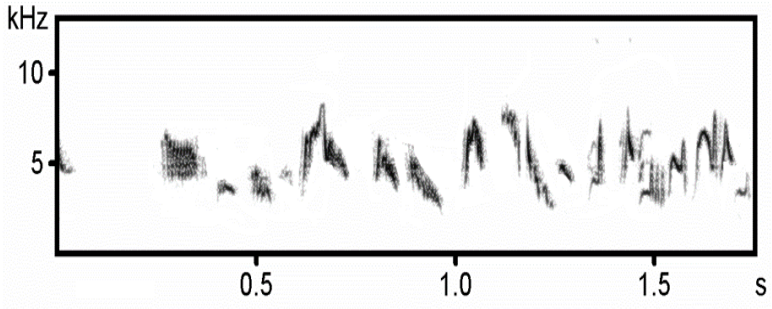


PA_1021-2

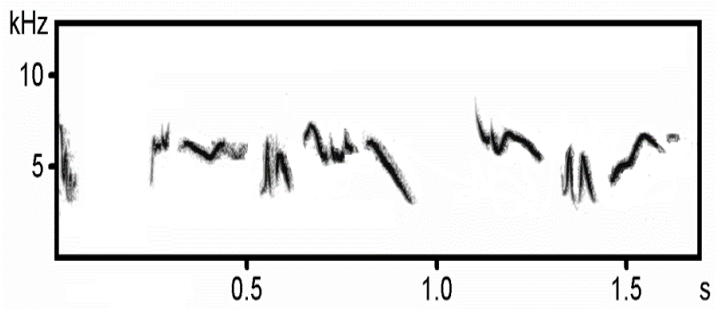
C. alopex



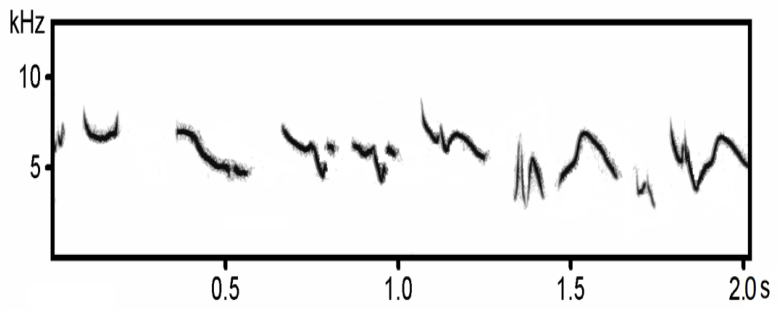
XC28606



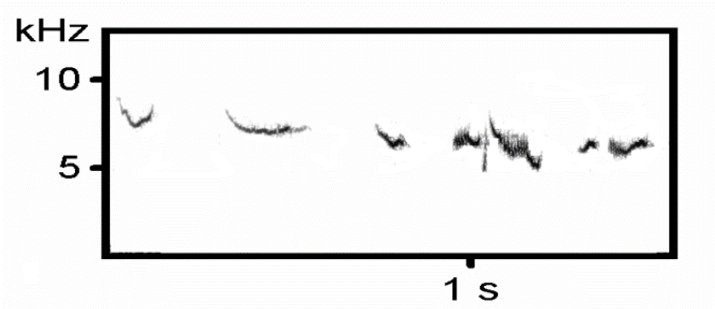
f. XC300042



XC300041

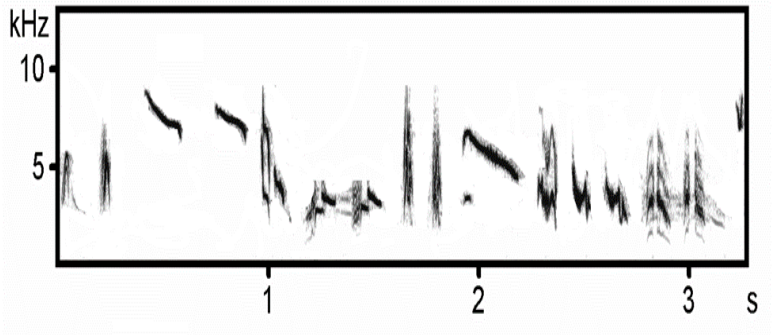


XC300043

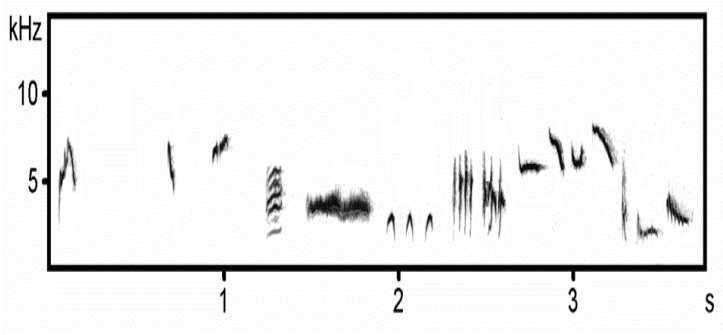


ML21270

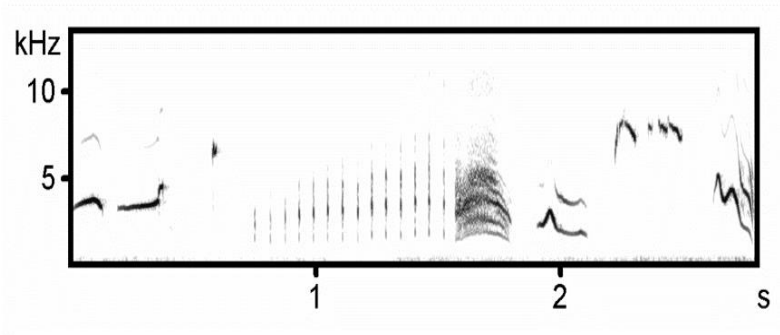
C. sabota



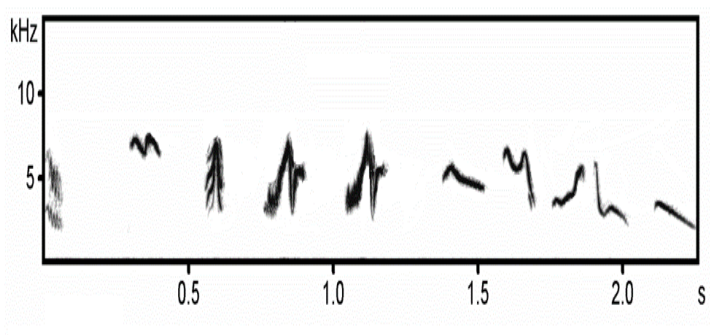
g. PA_2017-1090-1



PA_2017-1090-2



AV_O-67-1

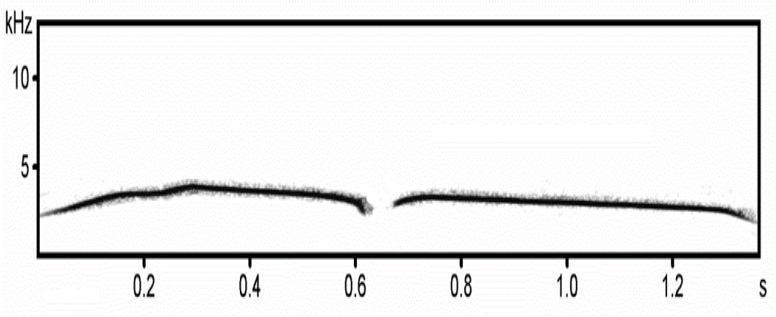


PA_2017-1095

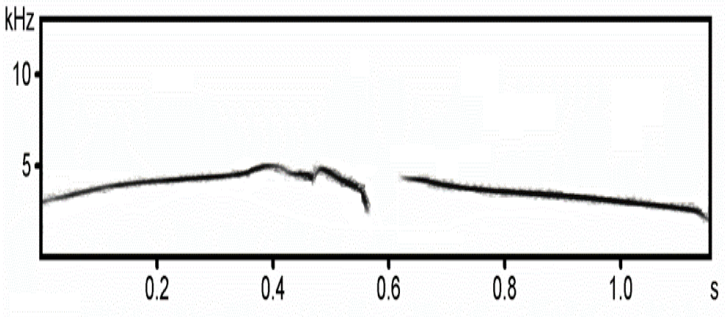
Certhilauda

Appendix 3.4.1 a-f

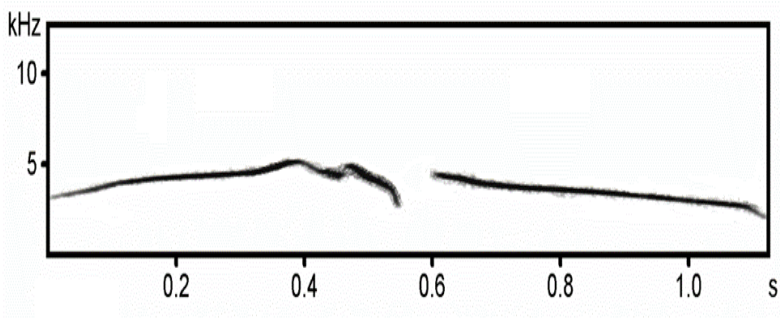
C. brevirostris



a. XC288868

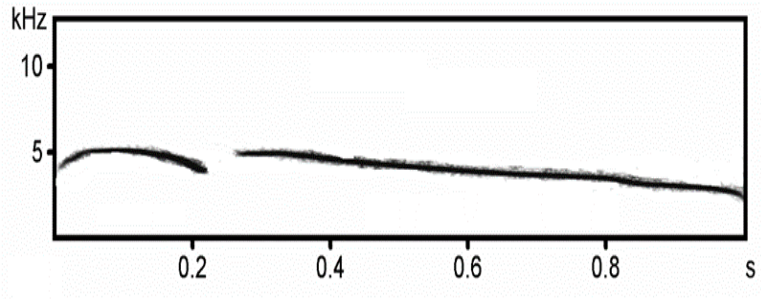


XC62564

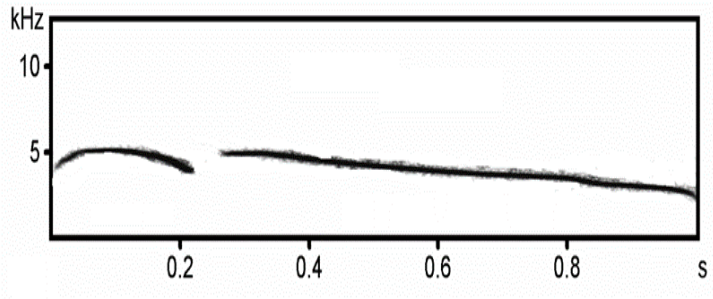


XC62562

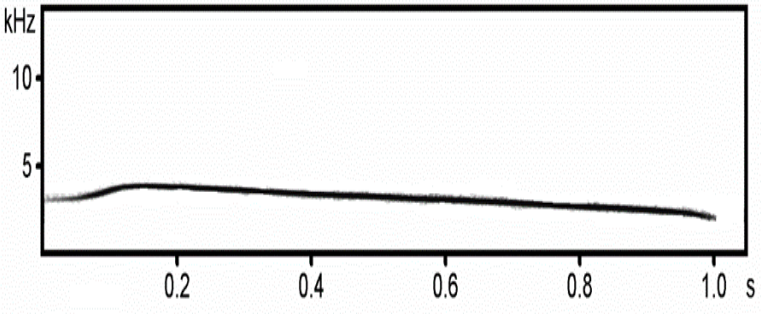
C. curvirostris



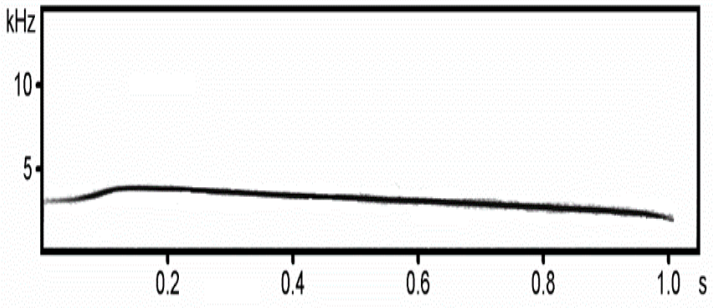
b. XC278147



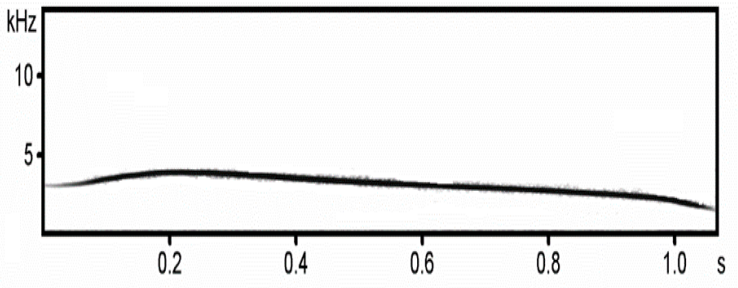
XC62554



PA_1011

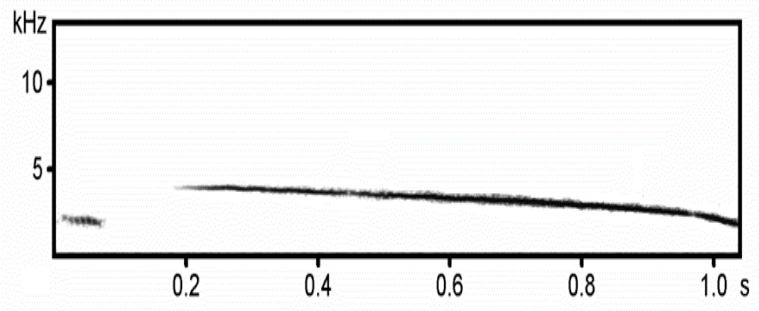


PA_1017

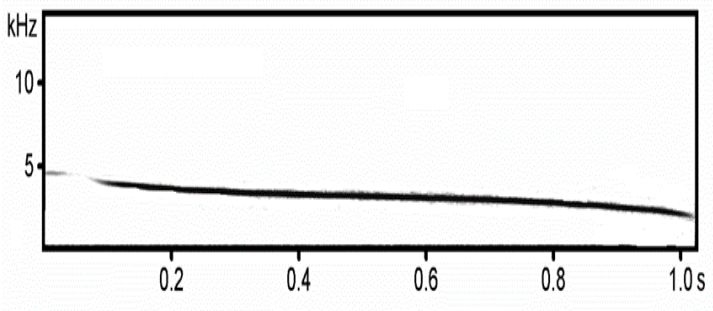


PA_1039

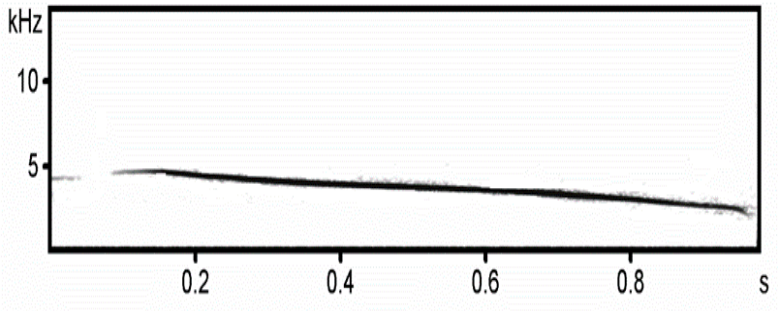
C. semitorquata



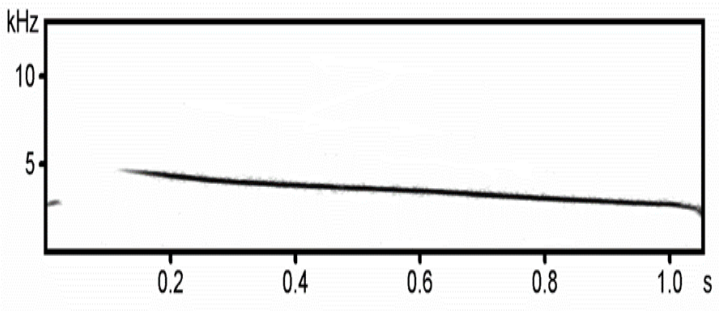
c. XC216682



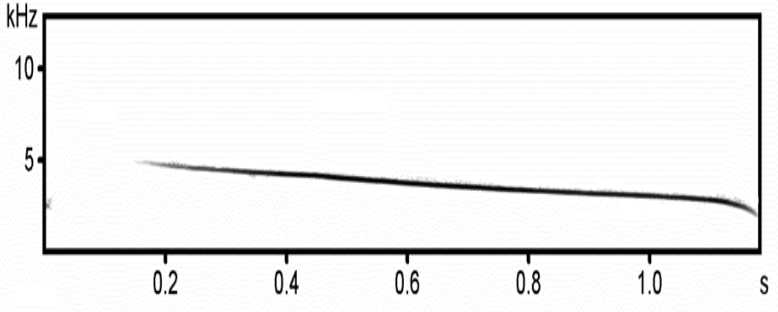
PA_2017-1065



PA_2017-1070

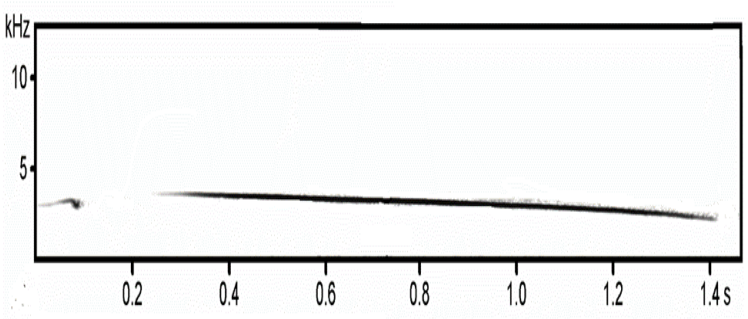


XC233912



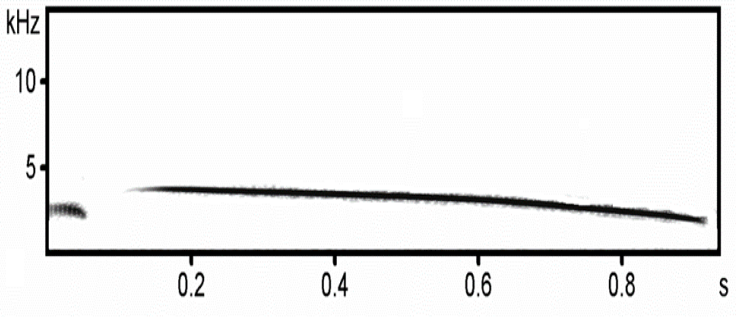
XC279991

C. benguelensis

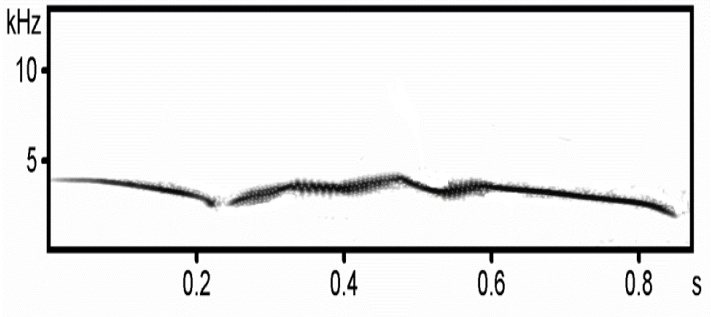


d. XC65245

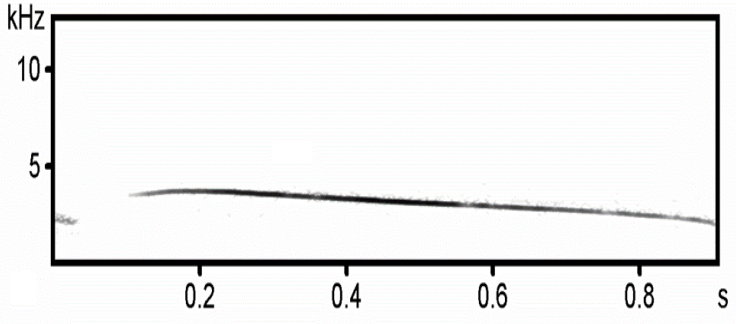
C. subcoronata



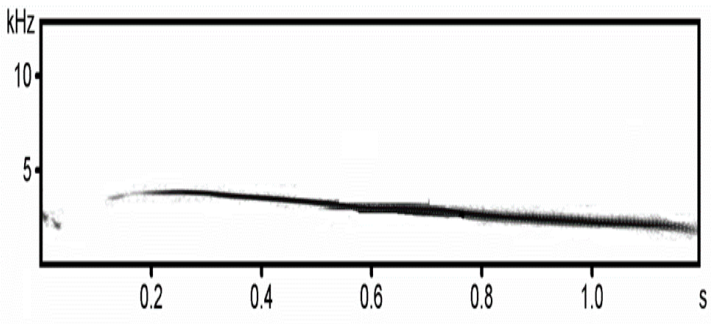
e. PA_1030



AV1-0-57-1

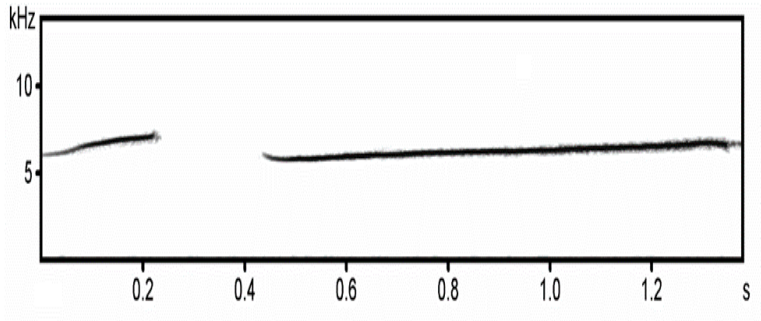


XC126492



XC146478

C. chuana

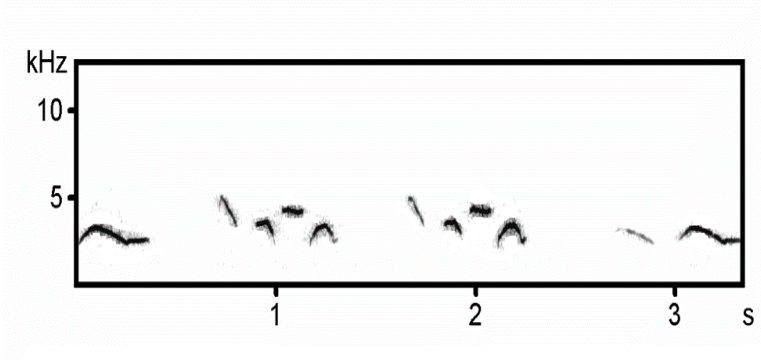


f. PA_-93-01-30

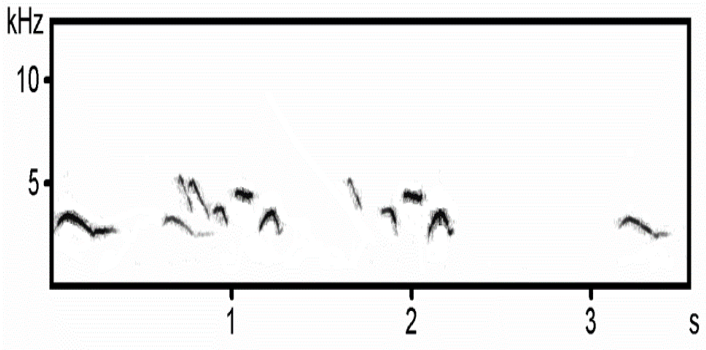
Eremopterix

Appendix 3.4.2 a-e

E. nigriceps

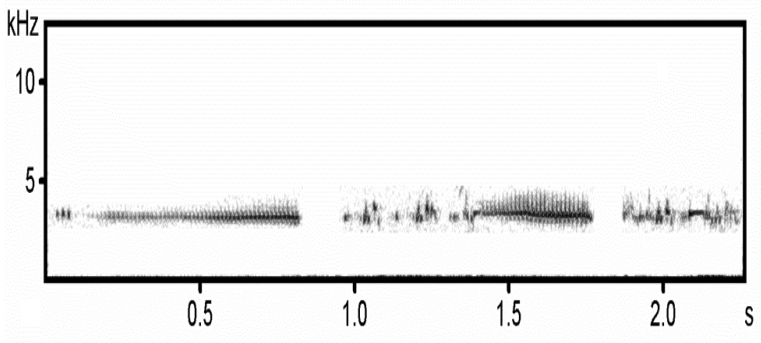


a. XC355815



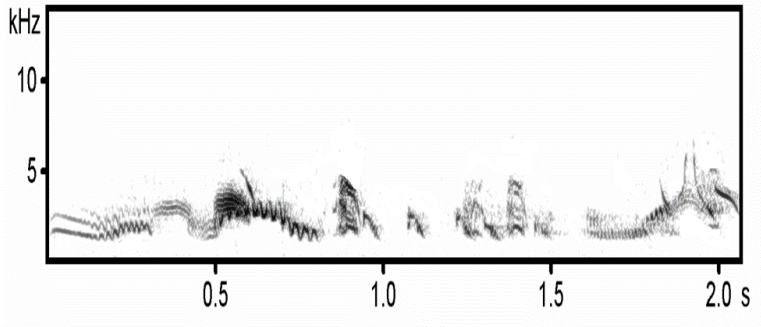
XC355816

E. griseus



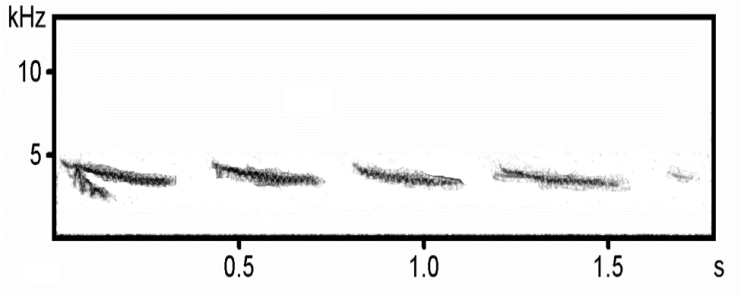
b. XC86569

E. signatus



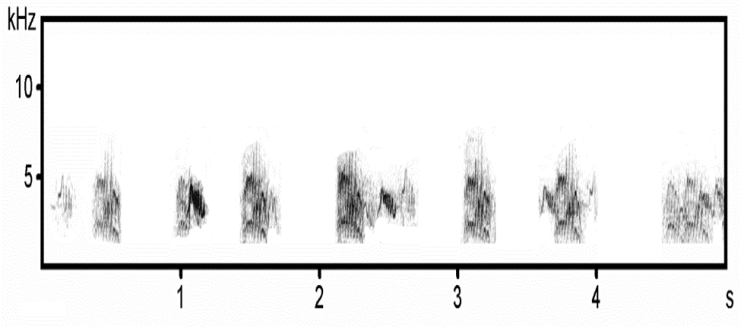
c. XC209981

E. leucotis



d. ML61186

E. verticalis

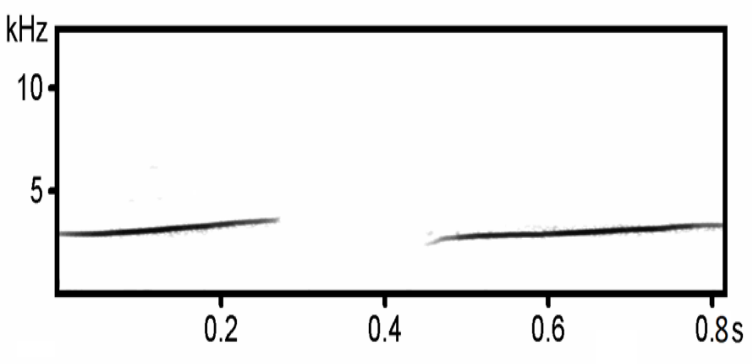


e. ML61158

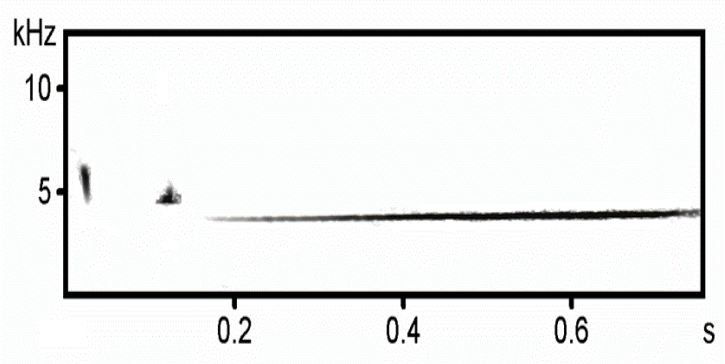
Ammomanes

Appendix 3.4.3 a-b

A. cintura

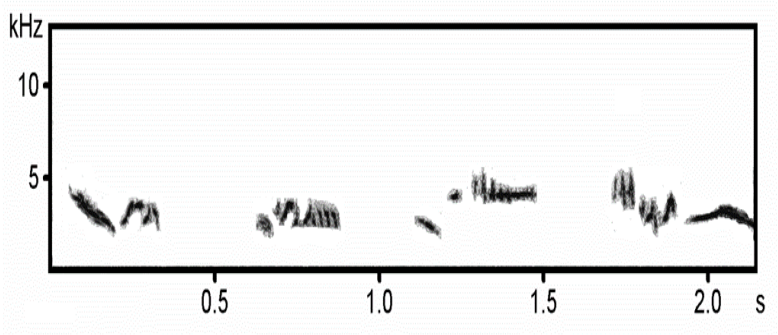


a. XC131914

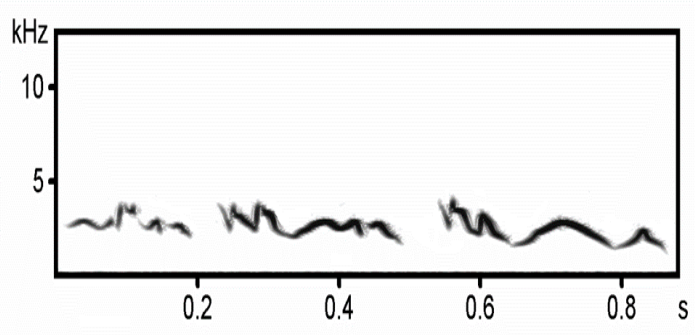


XC131913

A. deserti



a. ML1723

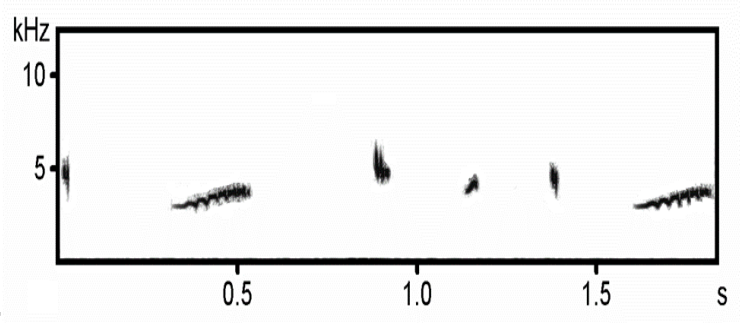


XC44494

Ramphocoris

Appendix 3.4.4 a

R. clotbey

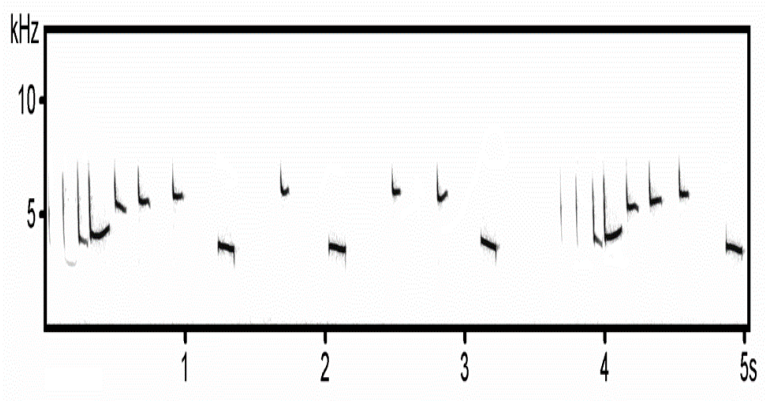


a. XC134442

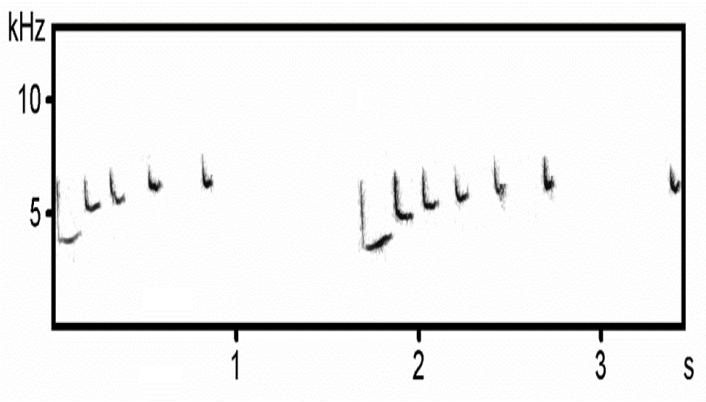
Ammomanopsis

Appendix 3.4.5 a

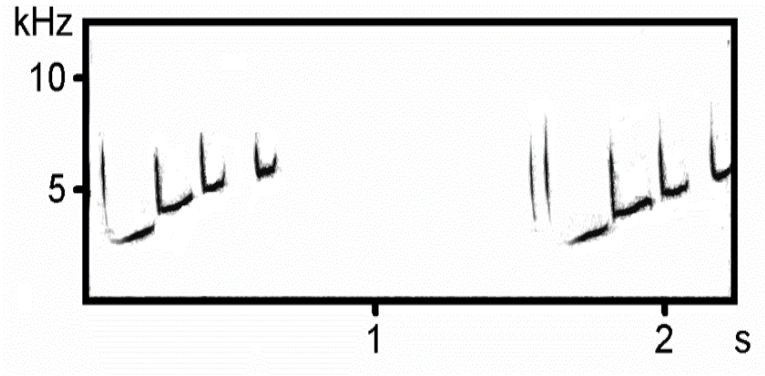
A. Grayi



a. ML61100



XC65276

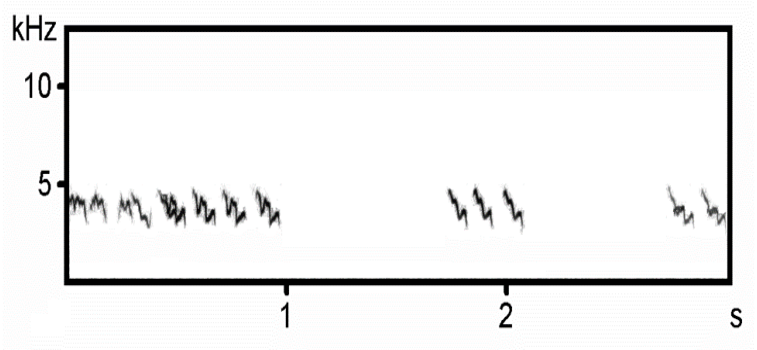


ML61107

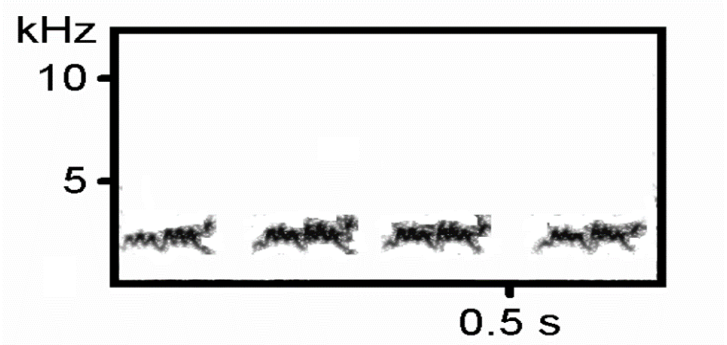
Chersomanes

Appendix 3.4.6 a

C. albofasciata



a. XC126330

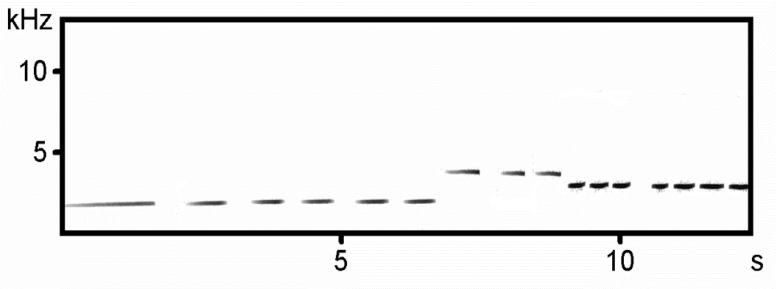


XC204237

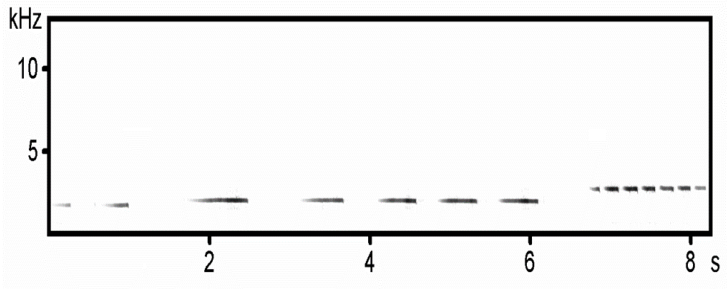
Alaemon

Appendix 3.4.7 a

A. alaudipes



a. XC164131



XC135148

CHAPTER 4

The evolution of vocal and syringeal characters traced on a phylogeny using a parsimony-based method

4.1 Background information

4.1.1 Ancestral state reconstruction and phylogenies

Ancestral state reconstruction is undoubtedly, a popular method used to extrapolate back in time from traits of individuals (or populations) to their common ancestors through mapping of traits onto molecular phylogenies (Simpson 2010, Joy *et al.* 2016). This method has been used to comprehend the progression of character evolution (Royer-Carenzi and Didier 2015), examination of variation in rates of diversification (Ricklefs 2007), mapping of ecological traits as well as re-evaluating past classifications (Tinberg 1959). Behavioural, morphological and ecological traits have been used in earlier studies strictly for phylogenetic inference (Lack 1947, Tinberg 1951), and variations that may arise in such characters can be inferred between organisms and their ancestors even if direct observations of those ancestors were not conducted (Maddison and Maddison 2000).

Challenges exist pertaining to methods used in ancestral state reconstructions and different approaches are used to address them, each with their own assumptions, advantages and shortcomings (Ho and Jermiin 2004). Some authors Royer-Carenzi and Didier (2015) maintained that based on the phylogeny of organisms and their characters, a reconstruction method should be able to derive the character states of ancestral organisms and related organisms. Thus, these can be analysed in the evolutionary framework specified by trees using any of the characters under study (Goldberg 2003). Furthermore, this should help systematists to present the biogeographic dispersal of species, test why and how characters evolved (Schaefer *et al.* 2012).

Two major classes of ancestral reconstruction methods have been proposed: parsimony-based principle (Maddison 1991; Collins *et al.* 1994), which assign the missing values of the tree by lessening the sum of distances between ancestors and their direct descendant characters, and also stochastic-based models of character evolution (Huelsenbeck and Ronquist 2001; Nielsen 2002). The stochastic approaches including maximum likelihood and Bayesian methods are considered advantageous over parsimony approach by some authors (Huelsenbeck *et al.* 2003, Ronquist 2004). This is due to their reliance on evolutionary models and that Bayesian method is used in studying character evolution while simultaneously accounting for both phylogenetic and mapping uncertainty (Ronquist 2004). However, they face the challenge that the ability to accurately reconstruct ancestral states deteriorates with increasing evolutionary time between a particular ancestor and its observed descendants.

Parsimony is generally used with the assumption that the tree being used is the true tree and that relevant taxa were included and characters were coded correctly. It takes the character as denoted on the character matrix while maximum likelihood maximises the probability that an observed state would evolve under a stochastic model of evolution (Pagel 1999). However, Fischer and Thatte (2010) maintained that if the upper bound on the substitution probabilities is small, every likelihood tree is also a parsimony tree (not vice versa). In some instances, similar results from parsimony and maximum likelihood ancestral state reconstructions were recovered (Helm *et al.* 2018).

What is common among these methods is that they are applied to an existing phylogeny (a tree-based hypothesis about the order in which taxa are related by descent from common ancestors) inferred from the same data (Joy *et al.* 2016). In other words, the ancestral state reconstruction methods assume that the given tree is congruent with the phylogeny on which the character evolved even though this may not always be true (Duchêne *et al.* 2015).

4.1.2 Study taxa

In this chapter, the focus was on birds commonly known as larks (Passeriformes, suborder Passeri, Alaudidae) (de Juana *et al.* 2020). They belong to a family which is comprised of 21 genera and 98 species (de Juana *et al.* 2020; Gill and Donsker 2020)

(Fig. 1.1). The distribution of larks is primarily of African descent, followed by Europe and Asia with one species dispersed in the New World (Horned Lark *Eremophila alpestris* native to North America) and some islands, with the Australian Bush Lark *Mirafra javanica* known to occur from south-east Asia to Australia. The distribution of larks is considered skewed, having two “hot-spots” of diversity (Barnes 2007). They match the arid zones of the south-west of Africa (Botswana, Namibia and South Africa) with 33 species (85% endemic) and north-east of Africa (Ethiopia, Kenya and Somalia) consisting of 37 species (62% endemic) (Barnes 2007). Larks occupy a wide range of heterogenous environments ranging from savanna, semi-arid and desert habitats (Alström *et al.* 2013).

Larks have been traditionally classified by bill morphology and plumage pattern (Keith *et al.* 1992, Donald 2004). With the introduction of molecular studies, the number of genera in the family had not been consistent with the current number being approximately 20 to 23 genera that represent Alaudidae (Sinclair and Ryan (2003); Donald 2004; Hockey *et al.* 2005; Barnes 2007; Alström *et al.* 2013). The hidden diversity and mix up in some taxa revealed by molecular and vocal data have seen the number of species increasing over the years (Alström 1998, Ryan *et al.* 1998, Ryan and Bloomer 1999, Guillaumet *et al.* 2005, Guillaumet *et al.* 2008, Alström *et al.* 2013).

The latest comprehensive phylogeny of larks was published in Alström *et al.* (2013), based on mitochondrial and nuclear sequence data and this yielded robust topology which pointed to three major clades, the Alaudid, Mirafrid and Ammomanid larks (Fig. 1.3). However, it was found that some relationships that were acknowledged within the family using morphological data were incongruent with the outcome of the molecular phylogeny. Furthermore, some relationships at clade and genus level were not supported with this being attributed to unavailability of sequence data for some species. This may have also led to incongruence between topologies inferred from different molecular markers.

In the work of Royer-Carenzi *et al.* (2013), results of when parsimony and maximum likelihood approaches were used in the reconstructions of ancestral character states were compared and found to be quite close. They maintained that performance heavily depends on the topology of the tree of taxa being studied, the ancestral node being inferred and the parameter values. In the work of Pedersen *et al.* (2007),

reconstructions of ancestral character states were performed under parsimony and Bayesian methods yielding reconstructions that were mostly congruent. This is despite Bayesian approach showing that the posterior probability of ancestral character states may decrease dramatically when node support was considered. Bayesian method also indicates that reconstructions may be ambiguous at internal nodes for highly polymorphic characters.

The reconstruction of ancestral character states requires that the taxa included in the phylogeny match those in the character matrix consisting of character states based on which reconstructions will be made. In the present chapter, there was a need to modify the known molecular phylogeny of larks in Alström *et al.* (2013) so that the taxa match those that form part of the vocal character matrix.

4.1.3 Aim

The aim was to reconstruct the molecular and vocal phylogenies of larks and also assess the evolution of vocal and syringeal characters traced on a modified or pruned molecular phylogeny of larks.

4.1.4 Objectives

The objectives were set out to:

i) generate a modified or pruned molecular phylogeny of lark species.

ii) reconstruct a vocal phylogeny of larks.

ii) reconstruct the ancestral vocal and syringeal character states of larks.

iii) determine if there are any defining characters for defined major clades (A – Alaudid, B – Mirafrid, C – Ammomanid) in within Alaudidae.

4.2 Materials and methods

4.2.1 Taxon and character sampling

4.2.1.1 Molecular and vocal phylogeny

Despite the availability of a comprehensive molecular phylogeny of larks, in this study, the phylogeny in Alström *et al.* (2013) could not be used as published. As per the aim in this chapter, the evolution of vocal and syringeal characters was to be traced and the same species for which song and syringeal characters were generated needed to be analysed in reconstructing lark molecular phylogeny. The combined DNA sequence dataset consisting of 81 lark species analysed in Alström *et al.* (2013) was provided and DNA sequences for 59 lark species (ingroup taxa) were extracted (Table 4.1). None of the DNA sequences were generated in this study. Few sequences were sourced from GenBank (Table 4.1) and added to Alström *et al.* (2013) dataset. The selection of outgroup taxa for the larks remains a challenge but literature was followed for guidance towards the choice of outgroups (Barnes 2007, Alström *et al.* 2013).

The combined sequence data analysed spanned five molecular markers, two mitochondrial (mt) and three nuclear (nc) markers. The five loci were: mt cytochrome *b* gene and part of the flanking rRNA – Thr (referred to as cyt*b*) which is ≤1002 base pair (bp) long, 16S rRNA (≤1016 bp); nc ornithine decarboxylase (ODC) exon 6 (partial), intron 6, exon 7, intron 7 and exon 8 (partial) (≤712 bp); the entire myoglobin (myo) intron 2 (≤729 bp) and the nuclear recombination activation gene, parts 1 and 2 (RAG) which is ≤2878 bp long.

For the vocal phylogeny, a matrix consisted of 69 ingroup species and similarly, *Cisticola* and *Prinia* species were considered as outgroups (Table 4.2).

4.2.1.2 Ancestral state reconstructions

Gross morphological and histological evidence as well as vocal evidence generated respectively from the syringes and songs strophes of larks and outgroup taxa in Chapter 2 (Table 2.1 and Table 2.2) and Chapter 3 (Table 3.2) were used in this chapter. Table 4.2 and 4.3 shows how vocal and syringeal character states were scored. The detailed

procedure in terms of how these character matrices were generated was outlined under the methods sections in the respective chapters. The following eight vocal characters and states were mapped: strophe length (short, intermediate, long), general strophe pitch (descending, ascending, stable), strophe type (aurally: musical, predominantly harsh), grouped-element ending (absent, present), grouped element ending structure (not applicable, warbling/bubbling, trilling), wing clapping (absent, present), wing clapping incorporation in strophe (not applicable - applies to the species where wing clapping is non-existent, absent - refers to instances where wing clappings are independently inserted not interfering with the sound, present - refers to instances where wing clappings are inserted in a way which make them form part of the sound or simply interfere with the sound), mimicry (sourced from literature: unknown, known). From the syringeal matrix only five characters were mapped and these were: divided bronchial rings, oblique (muscular-like) structure on the ventral side, degree of bronchial ring ossification, bronchial ring ossification pattern and bronchial ring completeness.

4.2.2 Analysis

4.2.2.1 Phylogenetic reconstructions

A combined file of aligned DNA sequences consisted of a total of 61 taxa, 59 ingroup and two outgroup taxa of which *Prinia* sp. was used to root the tree. A combined analysis was followed as this was generally found to have yielded a well-resolved and well-supported phylogeny in Alström *et al.* (2013). The vocal character matrix analysis was comprised of 69 lark species. Both character matrices were imported in PAUP ver. 4.0b10 (Swofford 2002) and analysed through parsimony inference method.

The analytical settings involved search parameters for the tree which included full heuristic search with all characters being unordered and with equal weight. The starting tree(s) was obtained via stepwise addition, tree-bisection-reconnection branch-swapping option and 1000 random additions of taxa (Maddison 1991) were in effect. Only one tree was held at each step during stepwise addition, with branches collapsed (creating polytomies) if the maximum branch-length was zero. Two multiple, equally parsimonious cladograms were recovered, and a strict consensus cladogram was constructed. To

evaluate the extent of each nodal support, bootstrap resampling (Felsenstein 1985) procedure with 1000 pseudo-replicates and five random additions of taxa per bootstrap pseudo-replicate was used.

4.2.2.2 Ancestral state reconstructions

The ancestral state reconstructions of eight vocal and five syringeal character states (Table 4.3) were made on the generated modified combined DNA phylogeny. Reconstructions were made using parsimony approach implemented in Mesquite ver. 3.2 (Maddison and Maddison 2017). All character states were unordered.

4.3 Results

4.3.1 Molecular and vocal phylogenetic outcomes

The combined mitochondrial and nuclear loci data set comprised 6 408 characters, out of which 905 were parsimony informative. The strict consensus parsimony phylogeny generated from two trees yielded a topology similar to the one published in Alström *et al.* (2013) (Fig. 4.1). The three major clades (A – the Alaudid, B - Mirafrid and C - Ammomanid) were recovered with strong nodal support (Fig. 4.1). Clade C is a basal clade to clade A and B which share a sister relationship. Alaudidae is supported with bootstrap support (BS) of 99%, clade A with 98%, the sister clades (A and B) have 81% BS (with each being supported with 98 and 82% BS respectively) and Clade C (60%). Although we analysed somewhat lesser ingroup taxa in this study compared to the number of taxa analysed in Alström *et al.* (2013), the parsimony phylogeny produced in this study has all the species placed in the same clades as in Alström *et al.* (2013). Therefore, the topology was similar, and the phylogeny was satisfactory for character tracing.

The vocal dataset comprised of eight characters with all of them being parsimony informative. The strict consensus tree was reconstructed from 1 222 most parsimonious trees and the phylogeny yielded wholly unresolved topology (Fig. 4.2). This means that no phylogenetic relationship can be inferred from song strophes of larks.

4.3.2 Ancestral state reconstructions

4.3.2.1 Wing clapping and wing clapping incorporation in song strophe

Among the larks, wing clapping has independently evolved only in clade B specifically in genus *Mirafra* but in selected species (*M. africana, M. fasciolata, M. apiata* and *M. rufocinnamomea*) (Fig. 4.3). However, the results are inconclusive concerning whether the most recent common ancestor had wing clappings or not in its song strophes. Despite the inconclusiveness of the actual state in the immediate ancestor of the genus *Mirafra*, wing clappings were present in the immediate ancestor of Alaudidae, *Cisticola* sp. (*Cisticola lais*) and absent in the earliest ancestor *Prinia* sp. This probably pointing to a possibility of the presence of wing clappings in some *Mirafra* spp. being a reversal state. This possibly being lost in clade A and a basal C and regained in clade B.

While selected *Mirafra* spp. and a *Cisticola* sp. were found to have evolved wing clapping in their song strophes, there is a difference in terms of how they are present in their strophes that is, whether they are incorporated or embedded directly in the strophe or appear as stand-alone but still in association with the strophe rendered. In Figure 4.4, wing clappings are incorporated directly in the strophe in *M. fasciolata* and *M. apiata* while in *M. africana, M. rufocinnamomea* and also *Cisticola* sp. appear as stand-alone but still being associated with the strophes.

4.3.2.2 Grouped element-ending and Grouped element-ending structure

The character ‘grouped element ending’ simply indicates that the strophe ends with elements that are rendered in groups and these grouped elements follow a particular structure that is, they may be rendered as a warble or a trill (fast or slow trill). What is prevalent among larks is the independent evolution of song strophes that end with grouped elements across the two terminal clades (A and B) and none in the basal clade C (Fig. 4.5). In clade A, this is observed in genus *Galerida; Eremophila alpestris; Alaudala raytal, A. rufescens* and *Melanocorypha maxima, M. yeltoniensis, M. calandra*. In clade B, this state is observed in *Mirafra microptera, M. javanica* and *Calendulauda barlowi, C. erythrochlamys, C. albescens* and *C. burra*. While this observation was absent in *Prinia* sp. it was present in *Cisticola* sp.

Even though it may be inconclusive regarding the presence of the grouped elements, the absence of grouped element-ending in strophes seems to be a widely distributed state within Alaudidae but being absent in the earlier ancestor (*Prinia* sp.). Based on this state being present in immediate ancestor of Alaudidae, there is a possibility that the presence of grouped elements could be a reversal state being lost in the basal clade C and regained in the terminal clade A and B (Fig. 4.6).

The structure of the grouped elements is a warble in *Galerida magnirostris, G. theklae* whereas *G. cristata* has a trill. *Alaudala raytal* and *A. rufescens* have a warble too while *E. alpestris, M. maxima, M. calandra* have a trill. *Mirafra microptera* and *M. javanica* have a warble.

4.3.2.3 Strophe type and strophe length

The character 'strophe type' reveals whether the strophe is predominantly musical (sometimes with harmonics) or predominantly screeching which means that it has trills (slow or fast). Figure 4.7 reveals that the earliest ancestor strophe was predominantly musical while the predominantly screeching (indicating the presence of trill) state evolved 11 times across Alaudidae in selected genera *Spizocorys, Alaudala, Melanocorypha* (clade A), *Mirafra* (clade B) and *Eremopterix* (clade C). It also appeared in *Cisticola* sp. The state 'predominantly musical' is plesiomorphic.

The strophe length has three states which are short (≤ 4 s), intermediate (4.1 – 8 s) and long (> 8 s) (Fig. 4.8). The short strophe is a plesiomorphic state with independent evolution of long strophes in clade A (*Alaudala rufescens* and *Melanocorypha maxima*) and clade C (*Alaemon alaudipes*).

4.3.2.4 Strophe pitch

The ancestral state for larks is ascending pitch in their strophes (Fig. 4.9). The re-emergence of stable strophe pitch in *Heteromirafra ruddi* and *Mirafra cheniana* in clade B which was present in the earliest ancestor *Prinia* sp. could be a case of convergent evolution. However, stable strophe pitch in the rest of genus *Spizocorys* in clade A is a synapomorphy.

4.3.2.5 Mimicry

Mimicry was coded as confirmed in literature. Mimicry is a form of vocal learning which involves copying of conspecifics, heterospecifics and other sounds (Kelley *et al.* 2008). It is inconclusive as to whether the ancestor of larks could mimic the songs of other birds or not hence it is not known what the lark ancestor could have been (Fig. 4.10). However, there is no known evidence of lark species in clade C imitating the songs of other species while mimicry has been observed in clade A and B in some species across the two clades or genera.

Contrary to what was reflected in literature that mimicry is absent in clade C (Ammomanid) (Barnes 2007), Engelbrecht and Dikgale (2017) presented evidence of heterospecific mimicry in *Eremopterix leucotis* song. Unfortunately, DNA sequence for *E. leucotis* was not available hence its absence in the phylogeny.

4.3.2.6 Degree of the syringeal divided bronchial ring and syringeal oblique structure

The evolution of divided bronchial ring was observed across all the clades in clade A (*Galerida magnirostris, Calandrella cinerea);* clade B *(Calendulauda africanoides, C. sabota)* and clade C *(Certhilauda curvirostris)* (Fig. 4. 11). This is absent in the outgroups. The ancestral state is absent despite the unavailability of syringeal information for most species.

Similarly, the oblique (muscle-like) structure on the ventral side of the syrinx was found to have evolved in *Chersomanes albofasciata* (Fig. 4.12) and not much can be deduced regarding its evolution.

4.3.2.7 Degree of bronchial ring ossification and bronchial ring ossification pattern

Despite the difficulty in tracing the evolution of these characters, what came out is that ossification of bronchial rings was found to be restricted to the centre of the bronchial rings across the major clades in *Calandrella cinerea, Mirafra cheniana, Calendulauda erythrochlamys, C. albescens, C. burra, C. africanoides, C. sabota, Certhilauda subcoronata* and *Chersomanes albofasciata*. On the other hand, ossification was found to be almost full that is, spanning almost the whole ring in *Galerida magnirostris, Spizocorys conirostris, S. sclateri, S. starki, Mirafra Africana, M. passerine, M.*

rufocinnamomea, Certhilauda curvirostris, Eremopterix verticalis as well as the outgroup *Cisticola* sp. (Fig. 4.13).

Figure 4.14 shows the pattern that ossification of bronchial rings takes. The non-serial pattern was observed in all the species that have ossification spanning almost the rest of the bronchial rings except for *Eremopterix verticalis* and *Cisticola* sp. The serial pattern was observed in all the species that have ossification being restricted to the centre of the bronchial rings with the addition of two species (except *Eremopterix verticalis* and *Cisticola* sp.) that have almost full ossification of the bronchial rings.

4.3.2.8 Bronchial ring completeness

The bronchial rings form a C-shape which makes the rings look almost closed/joined or open. The closed bronchial rings were observed in *Galerida magnirostris, Spizocorys conirostris, S. sclateri, S. starki, Mirafra Africana, M. passerine, M. fasciolata* and *M. curvirostris* whereas the open bronchial rings were found in *Calendulauda erythrochlamys, C. albescens, C. burra, C. africanoides, C. sabota*, *Certhilauda subcoronata, C. verticalis, Chersomanes albofasciata* and the outgroup *Cisticola* sp. (Fig. 4.15).

It is worthwhile to point out that despite *Cisticola* being an outgroup species, most of the states are not plesiomorphic.

4.4 Discussion and Conclusion

The tracing of the evolution of vocal and syringeal characters revealed that among the 13 characters for which the ancestral reconstructions were performed, 12 are more polymorphic that is, they underwent multiple state changes ranging from four to 18. Only one character, oblique muscular-like structure on the ventral side of the syrinx registered one change but this cannot be explained based on the fact that this character was found in only one syrinx of an individual bird.

About the earliest common ancestor

The earliest ancestor of Alaudidae predominantly had musical song strophes that were short characterised by stable pitch and lacked grouped element at the end. In addition,

the song strophes lacked wing clappings. With regard to mimicry, ambiguity of states at the ancestral node makes it difficult to determine whether the ancestor would mimic songs of other species or not. On account of the syringeal structure, the ancestor had divided bronchial rings that had almost fully ossified in a manner which gives a serial pattern. The bronchial rings were open, and the syrinx did not have a muscle-like oblique structure on the ventral side.

Evolution of characters within Alaudidae

The absence of wing clappings in song strophes of larks in clade A and C and all other genera in clade B is a plesiomorphic state present in the earliest and immediate ancestors of the major clades of study species while wing clappings are present in genus *Mirafra* albeit not in all species. The acquisition of wing clappings is unique and mainly restricted to the southern African *Mirafra* species. However, the uncertainties around the outgroups of larks complicate matters given that wing clappings are present in some *Cisticola* species (*Cisticola lais*). Seemingly, wing clappings is an adaptation mainly associated with moments of display sometimes when a bird takes off and at a time when a bird descends.

The absence of the grouped element at the end of the song strophes of ancestors of the three major clades is plesiomorphous while with the evolution of grouped element in song strophes of five genera across clade A and B are unique. However, this does not assist in explaining the evolution of this state since the relationship across these genera is mostly polyphyletic. The species that end their song strophes with grouped elements that is, either trilling or warbling thrive in shrubland to grassland habitats. Generally, these species have short elements and put more space in between elements to reduce reverberation and even though the song strophes end with trilled elements that are somewhat longer. The presence of trills and a number of spaced individual elements is an adaptation to open habitat. This affirmation makes it not a good character to map in a phylogeny, because is under selection pressure of habitat that could mask its evolution that is, there will be homoplasy between species from open habitats.

The strophe type in terms of the texture shows that some genera in all three clades have strophes that have acquired predominantly screeching state in which case they

possess fast tills anywhere along the strophes. However, the ancestral state leading to *Melanocorypha* and *Alaudala* is indecisive. Predominantly musical state is a plesiomorphous state.

With regard to the length of the song strophe, most species have short song strophes, and this is a primitive state. In clade A, *Alaudala rufescens* and *Melanocorypha maxima* and *Alaemon alaudipes* in clade C independently acquired a long state. Intermediate state has been acquired in all the clades, but the polyphyletic relationship renders this state meaningless.

As far as song strophe pitch is concerned, ascending pitch is ancestral at family level and clade level while the state in the earliest ancestral node is indecisive being either ascending or stable. The stable state in genus *Spizocorys* is synapomorphic and it is a state which agrees with the relationships in this group. The acquisition of stable pitch in clade B cannot be explained by the relationships between species in this clade and those in clade A and this could be attributable to homoplasy.

Despite that the earliest ancestors lacked mimicry, the presence of mimicry across the three clades does not help in tracing its evolution since its presence is ambiguous. Therefore, this character is of no value in tracing its evolution and in reconstructing the ancestral state in Alaudidae. The syringeal sampling coverage was poor because only species that are found in South Africa (or southern Africa) would be sampled and therefore, the representation for the clades was poor. From the reconstructions, the states at the nodes are indecisive.

In conclusion, most of the character states were found to plesiomorphous and mainly leading to clades of which their ancestral nodes were defined largely by autapormorphic and symplesiomorphic states. These do not assist in explaining how the various characters evolved. On the other hand, in case of song strophe pitch, a stable state in genus *Spizocorys* was found to be synapomorphic even though the acquisition of this state across Alaudidae may be homoplasious. The dispute about the relatives of larks also makes reconstruction of ancestral states challenging especially where the earliest ancestral nodes are defined by indecisive states. This in turn, render the explanation of reversals or states identified as autapomorphic and synapomorphic in this context up the

tree difficult. The question remains whether synapormorphic state in song strophe pitch can be considered a defining character for the larks. The presence of most non-plesiomorphic states in *Cisticola* which is an outgroup species is surprising. However, it is difficult to find an explanation towards this given the indecisiveness that exists regarding the relatives of the family Alaudidae.

Phylogenetically, while the molecular phylogeny was largely resolved, there is hardly phylogenetic signal in song strophes of larks.

Therefore, it is critical that there are decisive outgroups in reconstructing ancestral states because this also has a bearing on the states of characters as inferred in the deeper and shallow nodes of the tree. With regard to complete lack of phylogenetic structure based on vocalisations, it could probably be due to low number of characters. It should be preferred that the number of characters get close to the number of taxa analysed. Another reason could be that the songs are highly variable among different song strophes from a single individual and across song strophes from different individual birds belong to the same species. Multiple sampling of syringes especially for histological examination for most species and for vocalisation for some species should be achieved for any future work.

TABLE 4.1. LIST OF INGROUP AND OUTGROUP SPECIES TAXA ANALYSED IN PHYLOGENETIC RECONSTRUCTIONS. GENBANK ACCESSION NUMBERS ARE PROVIDED.

Taxon	Sample No. / Specimen No. / Reference	Locus				
		Cytochrome *b*	16S	ODC	Myoglobin	RAG
Galerida magnirostris	Tieleman *et al.* (2003) / PFP TL	AY165169	KF060396	-	-	-
Galerida theklae	PFP TkL (*P*)	KF060458	KF060397			
Galerida cristata	Guillaumet *et al.* (2006)	DQ028951	-	-	-	-
Calandrella cinerea	PFP CcinP119 / PFP RC2	KF060421	KF060358	KF060561	KF060507	KF060619
Calandrella acutirostris	DZUG U577	KF060412	–	KF060557	KF060504	–
Calandrella brachydactyla	DZUG U582 *(P)*	KF060416	–	–	–	–
Eremophila bilopha	Tieleman *et al.* (2003) / PFP THL / MNHN 2003-2732	AY165157	KF060360	KF060569	–	–
Eremophila alpestris	FMNH 351146 / DZUG U154	KF060446	KF060359	KF060568	KF060513	KF060625
Alauda leucoptera	DZUG U579 (*P*)	KF060463	–	KF060580	KF060525	KF060634
Chersophilus duponti	DZUG U2255	KF060441	–	KF060566	KF060512	KF060624
Alaudala cheleensis	DZUG U2202	KF060418	–	KF060560	KF060506	–
Alaudala rufescens	Tieleman *et al.* (2003) / PFP LST1	AY165154	KF060355	KF060563	KF060509	KF060620
Alaudala somalica	Tieleman *et al.* (2003) / PFP AST2	AY165166	KF152963	–	–	–
Alaudala raytal	DZUG 2201 (*P*)	KF060423	–	KF060562	KF060508	–
Melanocorypha maxima	DZUG U578 (*P*)	KF060464	–	KF060581	–	–
Melanocorypha bimaculata	DZUG U2283 (*P*) / DZUG U2283 (*P*)	KF060461	–	KF060578	KF060523	–
Melanocorypha yeltoniensis	DZUG U575 (*P*)	KF060467	–	KF060584	KF060528	KF060636
Melanocorypha calandra	MNHN 2003-2733	–	–	KF060579	KF060524	KF060633

Spizocorys conirostris	PFP P177	KF060495	KF060395	KF060607	–	KF060648
Spizocorys fringillaris	PFP P179	KF060496	KF060394	–	–	–
Spizocorys sclateri	Tieleman *et al.* (2003) / PFP P191	AY165170	KF060391	–	–	–
Spizocorys starki	Tieleman *et al.* (2003) / PFP P178	AY165162	KF060392	–	–	–
Lullula arborea	Tieleman *et al.* (2003) / PFP WL	AY165158	KF060356	KF060577	KF060522	KF060632
Mirafra africana	NMK RN1 / NMK RN1	KF060469	KF060389	KF060586	KF060530	–
Mirafra angolensis	PFP MA1	KF060470	–	KF060587	KF060531	KF060637
Mirafra cheniana	PFP P192	KF060474	KF060384	–	–	–
Mirafra hypermetra	NMK RWBL	KF060479	KF060387	–	–	–
Mirafra passerina	Tieleman *et al.* (2003) / PFP P186	AY165163	KF060383	KF060597	KF060540	KF060642
Mirafra rufocinnamomea	PFP FLTz	KF060486	KF060381	KF060599	KF060542	KF060644
Mirafra fasciolata	DZUG U2345 (*P*)	KF060477	–	KF060593	KF060537	–
Mirafra apiata	PFP P174	KC869741	KF060388	KF060588	KF060532	KF060638
Mirafra javanica	DZUG U3272	KF060480	–	KF060595	–	–
Mirafra microptera	DZUG U3275 (*P*) / DZUG U3276 (P)	KF060485	–	KF060596	KF060539	KF060641
Mirafra assamica	DZUG U3269 (*P*)	KF060471	–	KF060589	KF060533	–
Mirafra erythrocephala	DZUG U3270	KF060475	–	KF060591	KF060535	–
Mirafra affinis	DZUG U3268 (*P*)	KF060468	–	KF060585	KF060529	–
Mirafra cantillans	DZUG U3273 (*P*) / PFP SBL	KF060472	KF060386	KF060590	KF060534	–
Mirafra erythroptera	DZUG U3271 (*P*)	KF060476	–	KF060592	KF060536	KF060639
Heteromirafra ruddi	PFP L8	KC869742	KF060371	KF060576	KF060520	KF060631
Calendulauda barlowi	PFP Pi4	KF060428	KF060367	–	–	–
Calendulauda erythrochlamys	Tieleman *et al.* (2003) / PFP P-Dune	AY165167	KF060366	–	–	–
Calendulauda albescens	PFP Pi3	KF060426	KF060365	–	–	–
Calendulauda burra	PFP P119	KF060429	KF060364	KF060564	KF060510	KF060621
Calendulauda africanoides	PFP P175	KF060425	KF060370	–	–	–
Calendulauda sabota	Tieleman *et al.* (2003) / PFP P181	AY165172	KF060363	KF060600	KF060543	KF060645

Certhilauda brevirostris	PFP P215/L2	KF060434	KF060377	–	–	–
Certhilauda curvirostris	PFP P220/L7	KF060436	KF060379	–	–	–
Certhilauda semitorquata	PFP P214/L1	KF060437	KF060378	KF060565	KF060511	KF060622
Certhilauda benguelensis	PFP P204/L	KF060433	KF060376	–	–	–
Certhilauda subcoronata	PFP P219/L6	KF060438	KF060380	–	–	–
Certhilauda chuana	PFP P96	KF060435	KF060375	–	–	–
Eremopterix nigriceps	Tieleman *et al.* (2003) / PFP BCL / DZUG U2259	AY165149	KF060344	KF060573	KF060517	–
Eremopterix signatus	NMK CHSL	KF060455	KF060347	–	_	_
Eremopterix verticalis	Tieleman *et al.* (2003) / PFP P99	AY165164	KF060345	–	–	–
Ammomanes cintura	PFP BrTdLk1 (*P*) / MNHN 2003-2735	KF060405	KF060353	KF060552	KF060500	KF060612
Ammomanes deserti	Fregin *et al.* (2012) / VH A1592 (B0703)	JX236373	–	JX236460[2]	JX236343	JX236414
Ramphocorys clotbey	CEFE Rhcl1 / PFP CLOT1	KF060494	KF060350	KF060606	KF060548	KF060647
Ammomanopsis grayi	Tieleman *et al.* (2003) / PFP P94	AY165168	KF060374	KF060556	KF060503	KF060617
Chersomanes albofasciata	Johansson *et al.* (2007)	–	–	EU680716	EU680604	–
Alaemon alaudipes	PFP HpB4 / MNHN 2003-2729	KF060400	KF060343	KF060550	KF060498	KF060609
Cisticola Sp.	Cibois *et al.* (1999)	–	AF094670	–	–	–
Prinia Sp.	Barker (2004) (cyt*b*); Barker *et al.* (unpublished) (RAG); Cibois *et al.* (1999) (16S); Fregin *et al.* (2012) (ODC, myo)	AY352536	AF094647	JX236470	JX236364	AY319998

TABLE 4.2. A CHARACTER MATRIX SHOWING THE SCORES FOR THE CHARACTER STATES AS USED IN THE RECONSTRUCTION OF VOCAL PHYLOGENY (CHARACTER STATES 1 – 8). CHARACTERS 9 – 13 REPRESENT THE SYRINGEAL CHARACTERS USED IN THE RECONSTRUCTION OF ANCESTRAL CHARACTER STATES ONLY.

	Character state scores												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Galerida magnirostris	0	?	1	0	0	0	1	1	1	0	0	0	0
Galerida deva	0	?	0	?	0	0	0	1	?	?	?	?	?
Galerida theklae	0	?	1	0	0	0	0	1	?	?	?	?	?
Galerida malabarica	0	?	0	?	0	0	1	1	?	?	?	?	?
Galerida cristata	0	?	1	1	0	0	1	1	?	?	?	?	?
Galerida macrorhyncha	0	?	0	?	0	0	0	0	?	?	?	?	?
Calandrella cinerea	0	?	0	?	0	0	0	1	1	0	1	1	1
Calandrella acutirostris	0	?	0	?	0	0	1	1	?	?	?	?	?
Calandrella erlanger	0	?	0	?	0	0	0	1	?	?	?	?	?
Calandrella brachydactyla	0	?	0	?	0	0	0	1	?	?	?	?	?
Eremophila bilopha	0	?	0	?	0	0	1	0	?	?	?	?	?
Eremophila alpestris	0	?	1	1	0	0	1	0	?	?	?	?	?
Alauda leucoptera	0	?	0	?	0	0	0	1	?	?	?	?	?
Chersophilus duponti	0	?	0	?	0	1	1	0	?	?	?	?	?
Alaudala chellensis	0	?	1	0	0	2	1	1	?	?	?	?	?
Alaudala rufescens	0	?	1	0	0	2	1	1	?	?	?	?	?
Alaudala somalica	0	?	0	?	1	1	0	1	?	?	?	?	?
Alaudala raytal	0	?	1	0	0	1	0	1	?	?	?	?	?
Melanocorypha maxima	0	?	1	1	0	2	0	1	?	?	?	?	?
Melanocorypha bimaculata	0	?	0	?	1	0	1	1	?	?	?	?	?
Melanocorypha yeltoniensis	0	?	1	0	0	0	0	0	?	?	?	?	?
Melanocorypha calandra	0	?	1	1	1	1	1	1	?	?	?	?	?
Spizocorys conirostris	0	?	0	?	0	0	2	0	0	0	0	0	0
Spizocorys fringillaris	0	?	0	?	0	0	2	0	?	?	?	?	?
Spizocorys sclateri	0	?	0	?	1	0	2	0	0	0	0	0	0
Spizocorys starki	0	?	0	?	1	0	2	0	0	0	0	0	0
Lullula arborea	0	?	0	?	0	0	1	0	?	?	?	?	?
Mirafra africana	1	0	0	?	0	0	0	1	0	0	0	0	0

Mirafra angolensis	0	?	0	?	1	1	1	0	?	?	?	?	?
Mirafra cheniana	0	?	0	?	0	0	2	1	0	0	1	1	1
Mirafra gilletti	0	?	0	?	1	0	0	0	?	?	?	?	?
Mirafra pulpa	0	?	0	?	0	0	1	0	?	?	?	?	?
Mirafra hypermetra	0	?	0	?	0	0	1	1	?	?	?	?	?
Mirafra passerina	0	?	0	?	1	0	1	0	0	0	0	0	0
Mirafra rufocinnamomea	1	0	0	?	0	0	2	1	?	?	?	?	?
Mirafra fasciolata	1	1	0	?	0	0	1	1	0	0	0	0	0
Mirafra apiata	1	1	0	?	0	0	1	1	?	?	?	?	?
Mirafra javanica	0	?	1	0	0	0	1	1	?	?	?	?	?
Mirafra microptera	0	?	1	0	0	0	1	0	?	?	?	?	?
Mirafra assamica	0	?	0	?	1	0	1	1	?	?	?	?	?
Mirafra erythrocephala	0	?	0	?	0	1	1	0	?	?	?	?	?
Mirafra affinis	0	?	0	?	0	1	1	0	?	?	?	?	?
Mirafra cantillans	0	?	0	?	0	1	1	0	?	?	?	?	?
Mirafra erythroptera	0	?	0	?	0	1	1	0	?	?	?	?	?
Heteromirafra ruddi	0	?	0	?	0	0	2	0	?	?	?	?	?
Calendulauda barlowi	0	?	1	1	0	0	1	0	?	?	?	?	?
Calendulauda erythrochlamys	0	?	1	1	0	0	1	0	0	0	1	1	1
Calendulauda albescens	0	?	1	1	0	0	1	0	0	0	1	1	1
Calendulauda burra	0	?	1	1	0	0	0	0	0	0	1	1	1
Calendulauda africanoides	0	?	0	?	0	0	0	1	1	0	1	1	1
Calendulauda alopex	0	?	0	?	0	0	1	1	?	?	?	?	?
Calendulauda sabota	0	?	0	?	0	0	0	1	1	0	1	1	1
Certhilauda brevirostris	0	?	0	?	0	0	0	0	?	?	?	?	?
Certhilauda curvirostris	0	?	0	?	0	0	0	0	1	0	0	0	0
Certhilauda semitorquata	0	?	0	?	0	0	0	0	?	?	?	?	?
Certhilauda benguelensis	0	?	0	?	0	0	0	0	?	?	?	?	?
Certhilauda subcoronata	0	?	0	?	0	0	0	0	0	0	1	1	1
Certhilauda chuana	0	?	0	?	0	0	1	0	?	?	?	?	?
Eremopterix nigriceps	0	?	0	?	0	0	0	0	?	?	?	?	?
Eremopterix griseus	0	?	0	?	1	0	1	0	?	?	?	?	?
Eremopterix signatus	0	?	0	?	1	0	0	0	?	?	?	?	?
Eremopterix leucotis	0	?	0	?	0	0	1	1	0	0	1	1	1

Eremopterix verticalis	0	?	0	?	1	1	0	0	0	0	0	1	1
Ammomanes cintura	0	?	0	?	0	0	1	0	?	?	?	?	?
Ammomanes deserti	0	?	0	?	0	0	1	0	?	?	?	?	?
Ramphocorys clotbey	0	?	0	?	0	0	0	0	?	?	?	?	?
Ammomanopsis grayi	0	?	0	?	0	0	1	0	?	?	?	?	?
Chersomanes albofasciata	0	?	0	?	0	0	0	0	0	1	1	1	1
Alaemon alaudipes	0	?	0	?	0	2	1	0	?	?	?	?	?
Cisticola lais	1	0	1	2	1	0	1	1	?	?	?	?	?
Prinia crinigera	0	?	0	?	1	0	1	0	?	?	?	?	?

TABLE 4.3. THE VOCAL AND SYRINGEAL CHARACTERS AND THE SCORES OF THEIR RESPECTIVE CHARACTER STATES AS USED IN THE RECONSTRUCTIONS OF CHARACTER STATES.

Characters	Character states
1. Wing clapping	0 absent, 1 present
2. Wing clapping incorporation in song	? not applicable, 0 absent,1 present.
3. Grouped-element ending	0 absent, 1 present.
4. Grouped element ending structure	? not applicable, 0 warbling/bubbling, 1 trilling.
5. Strophe type (aurally)	0 predominantly tonal/musical, 1 predominantly harsh/screeching.
6. Strophe length	0 short, 1 intermediate, 2 long.
7. General strophe pitch	0 descending, 1 ascending, 2 stable.
8. Mimicry (Literature)	0 unknown, 1 known.
9. Divided/double bronchial rings	? not applicable, 0 absent, present
10. Oblique (muscle-like) structure on the ventral side	? not applicable, 0 absent, 1 present
11. Degree of bronchial ring ossification	? not applicable, 0 almost to full ossification, 1 restricted to the centre of bronchial rings
12. Bronchial ring ossification pattern	? not applicable, 0 non-serial, 1 serial pattern
13. Bronchial ring completeness	? not applicable, 0 almost closed/joined C-bronchial rings, 1 open C-bronchial rings

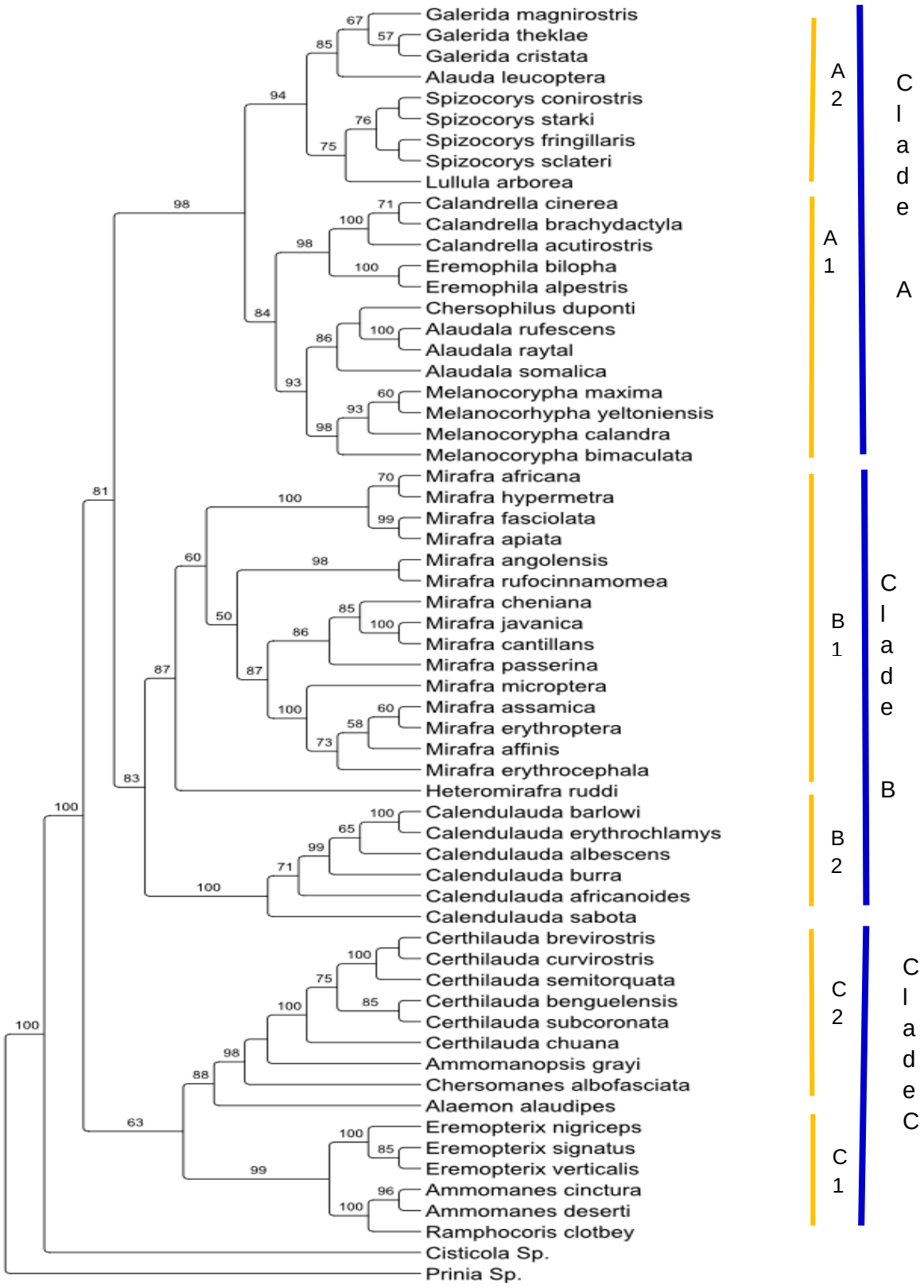


FIGURE 4.1. A STRICT CONSENSUS PRUNED PARSIMONY PHYLOGENY OF LARKS (ALAUDIDAE) GENERATED FROM COMBINED MITOCHONDRIAL AND NUCLEAR DNA SEQUENCE CHARACTERS. NUMBERS SHOWN ABOVE BRANCHES ARE BOOTSTRAP VALUES (ONLY ≥ 50%).

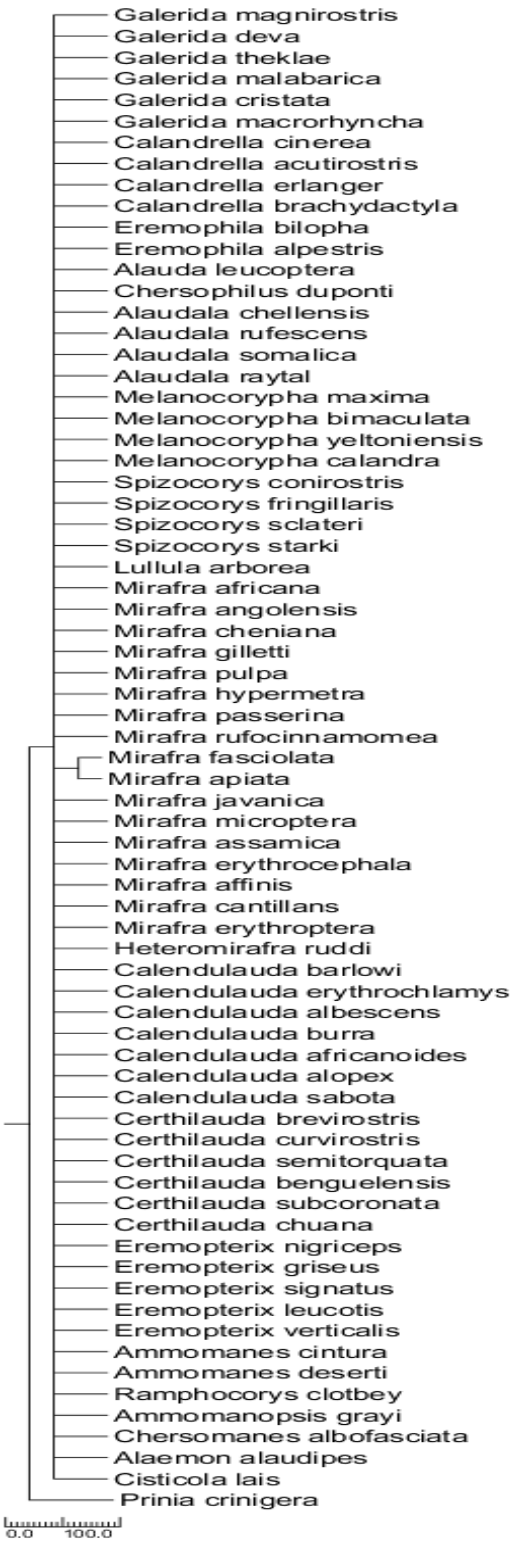


FIGURE 4.2. A STRICT CONSENSUS PARSIMONY PHYLOGENY OF LARKS (ALAUDIDAE) RECONSTRUCTED FROM VOCAL CHARACTERS.

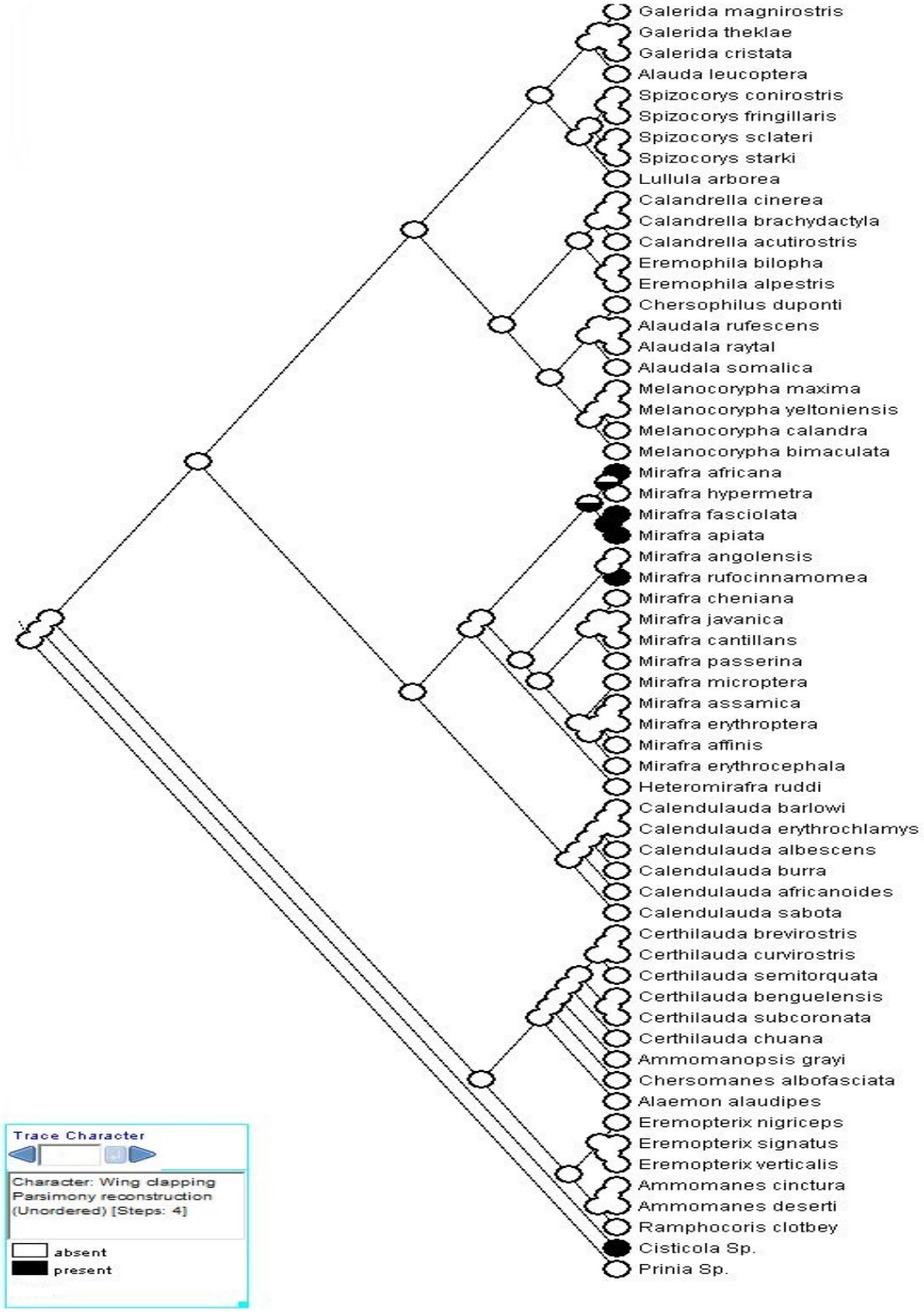


FIGURE 4.3. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF WING CLAPPING TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

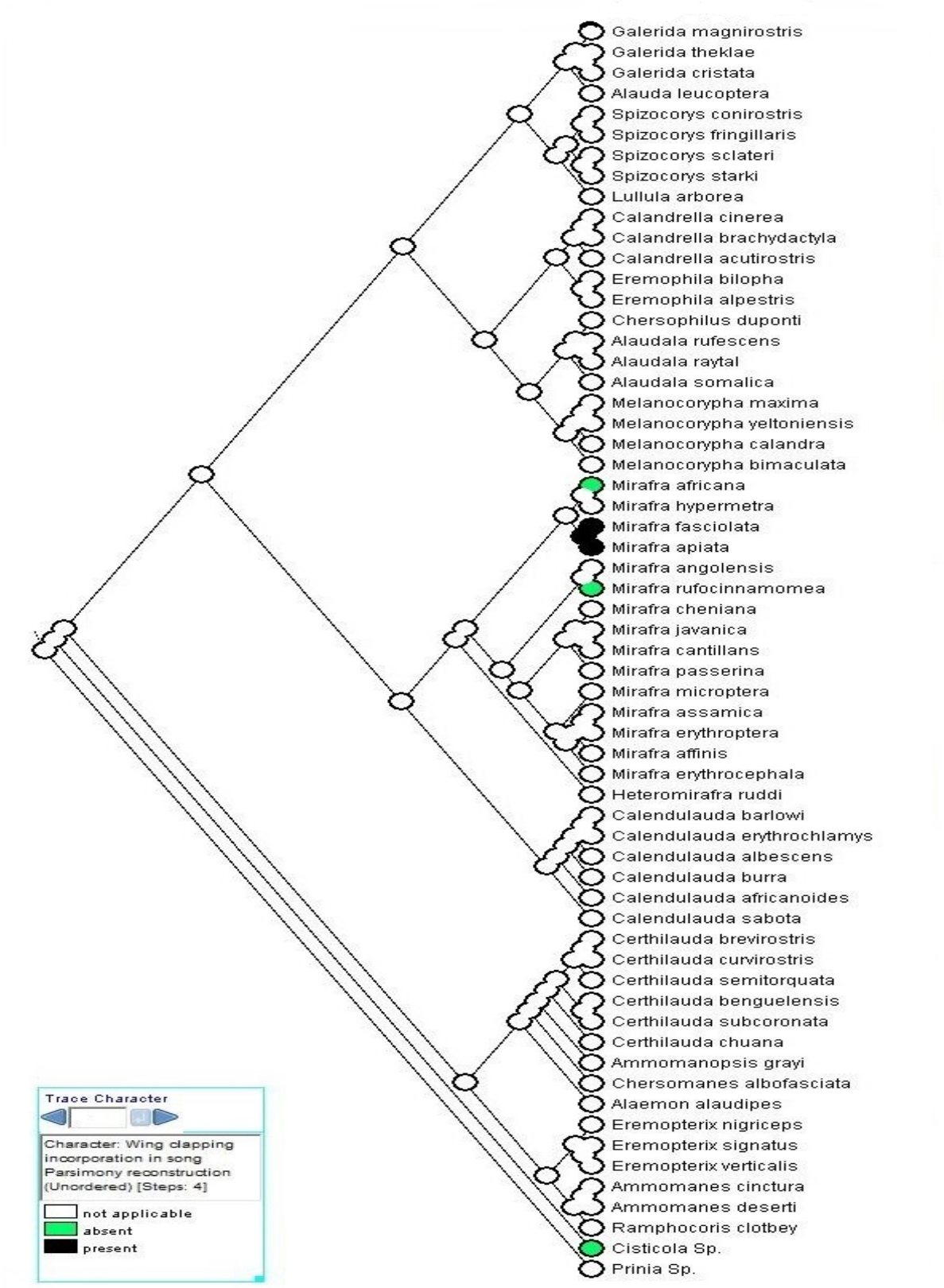


FIGURE 4.4. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF WING CLAPPING INCORPORATION IN SONG TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

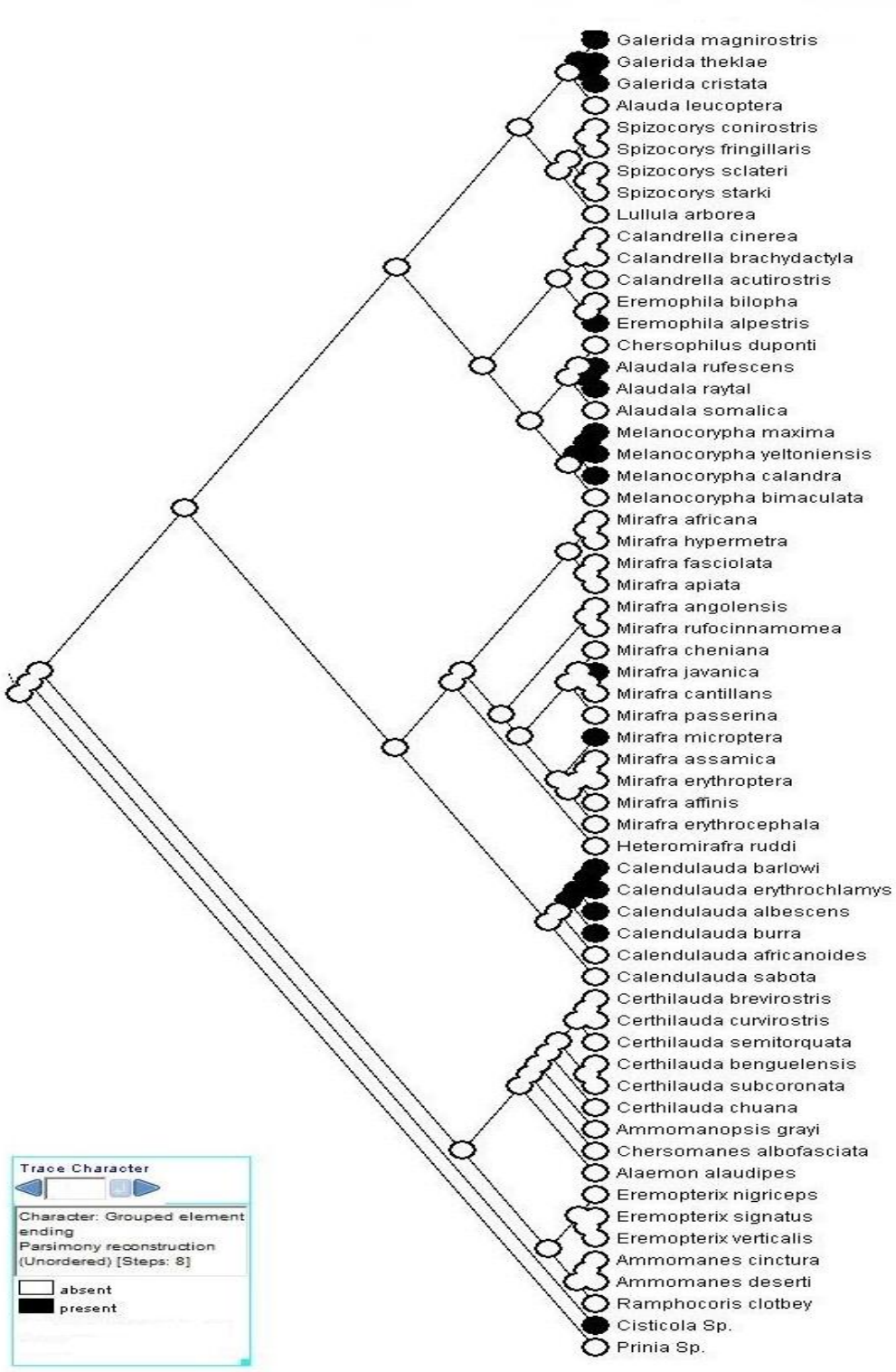


FIGURE 4.5. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF GROUPED ELEMENT-ENDING TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

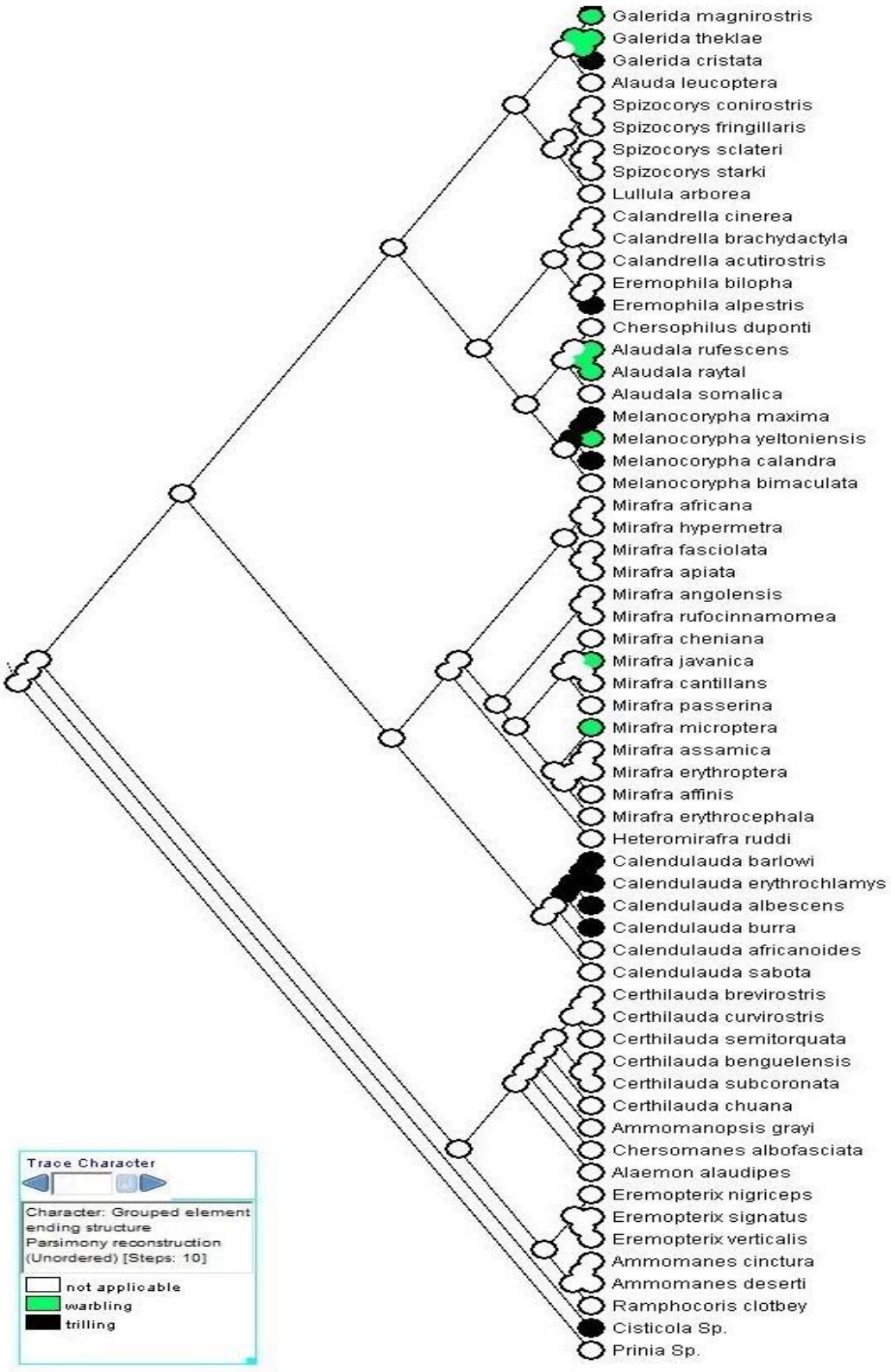


FIGURE 4.6. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF GROUPED ELEMENT-ENDING STRUCTURE TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

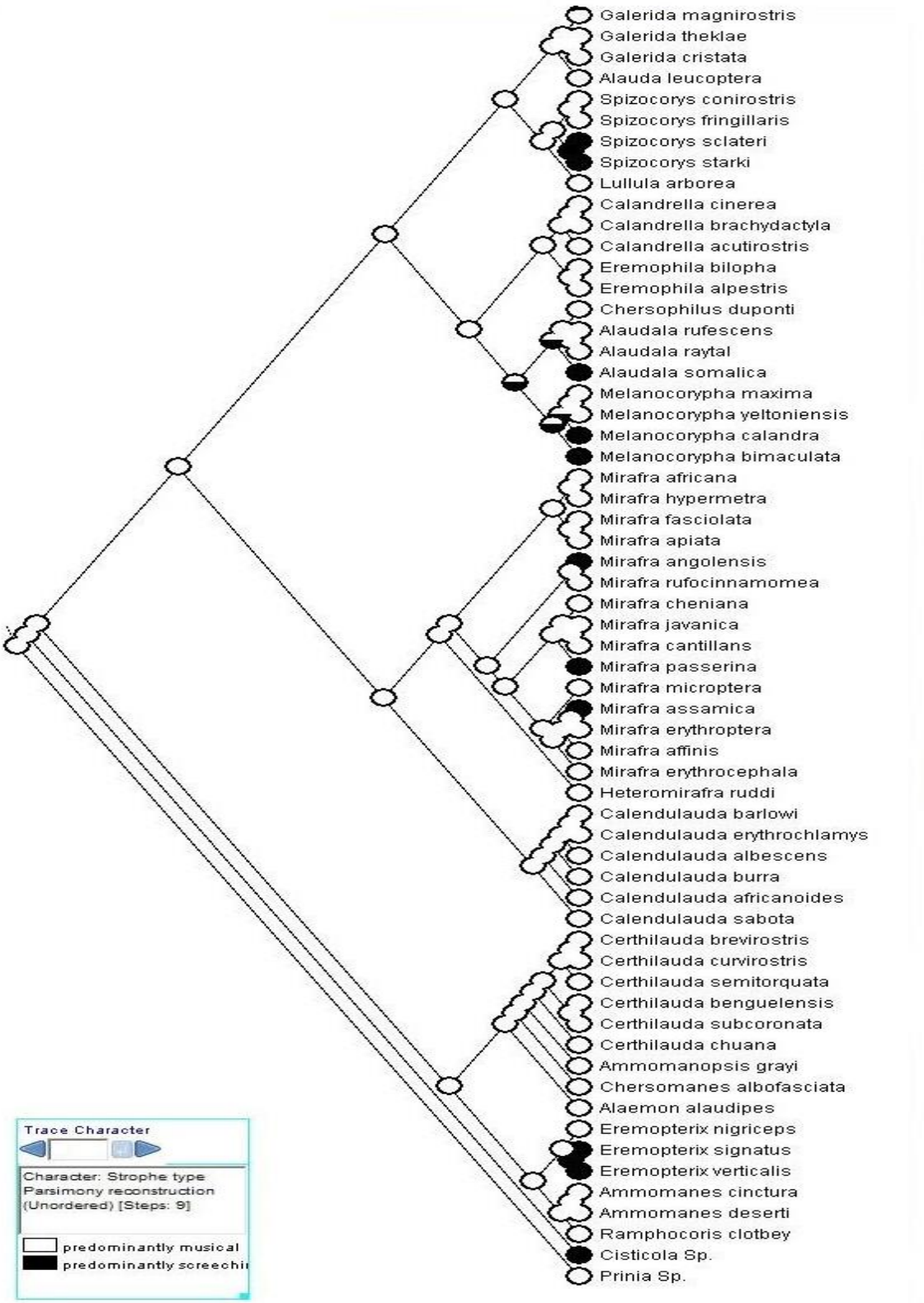


FIGURE 4.7. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF STROPHE TYPE TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

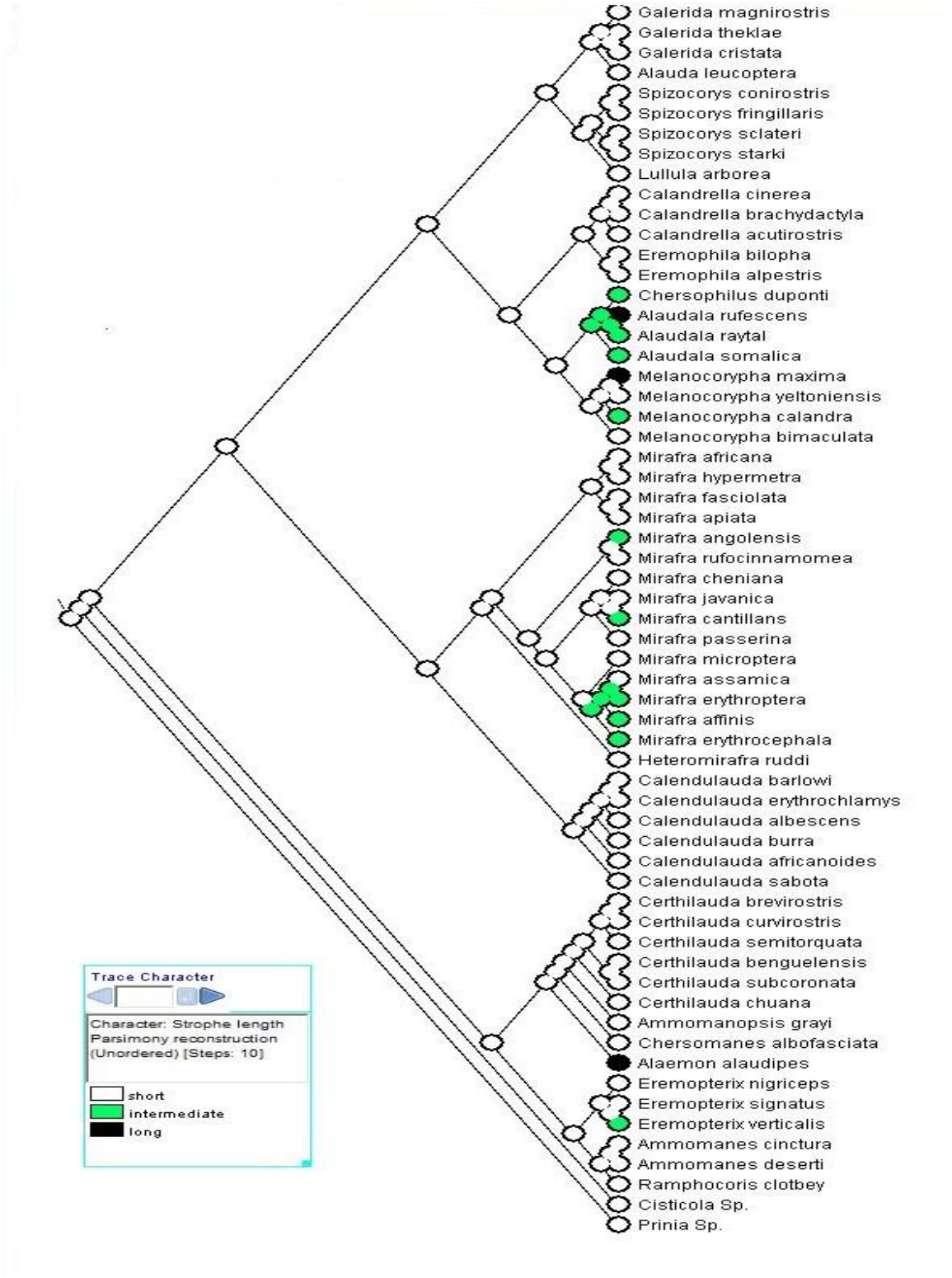


FIGURE 4.8. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF STROPHE LENGTH TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

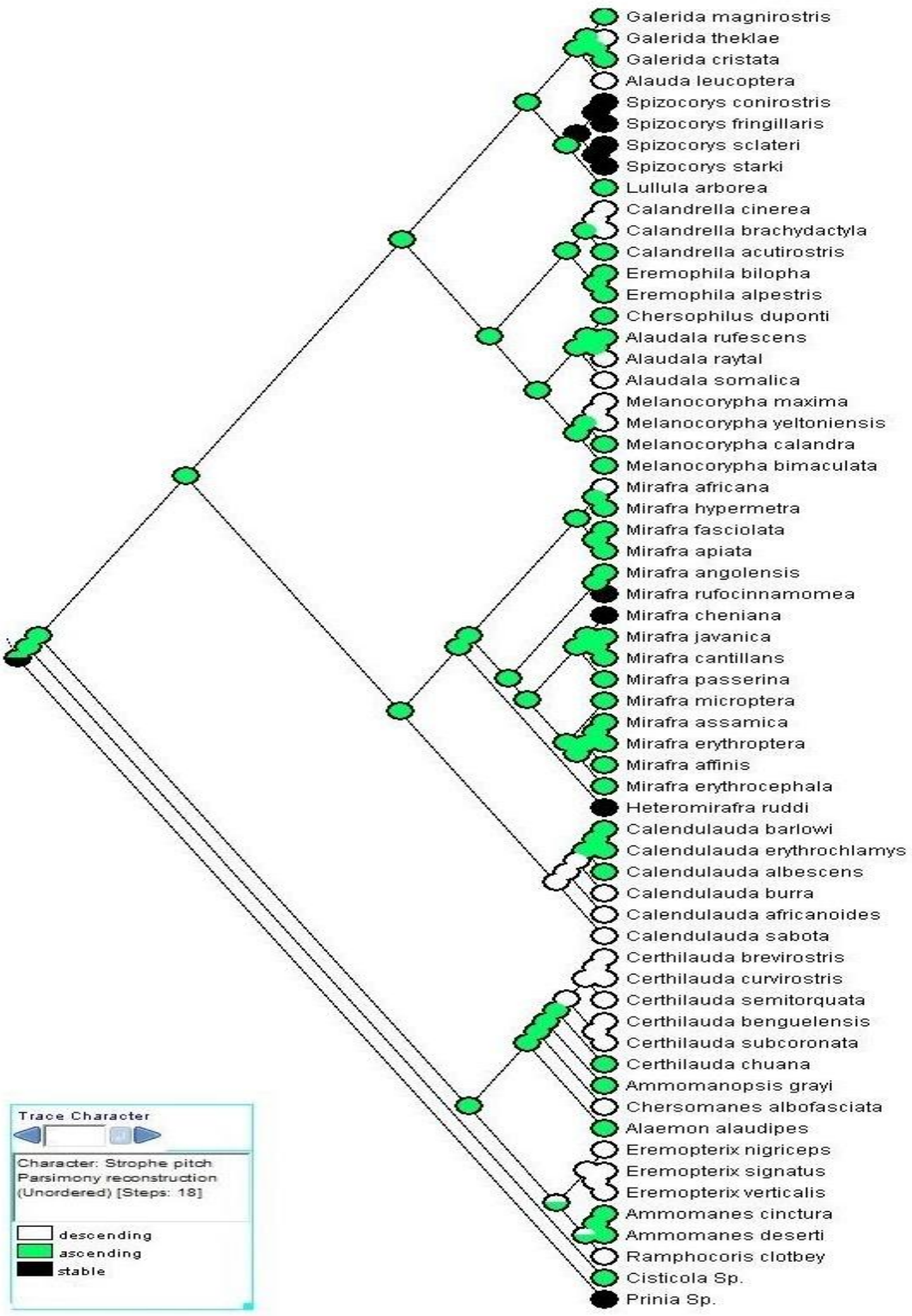


FIGURE 4.9. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF STROPHE PITCH TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

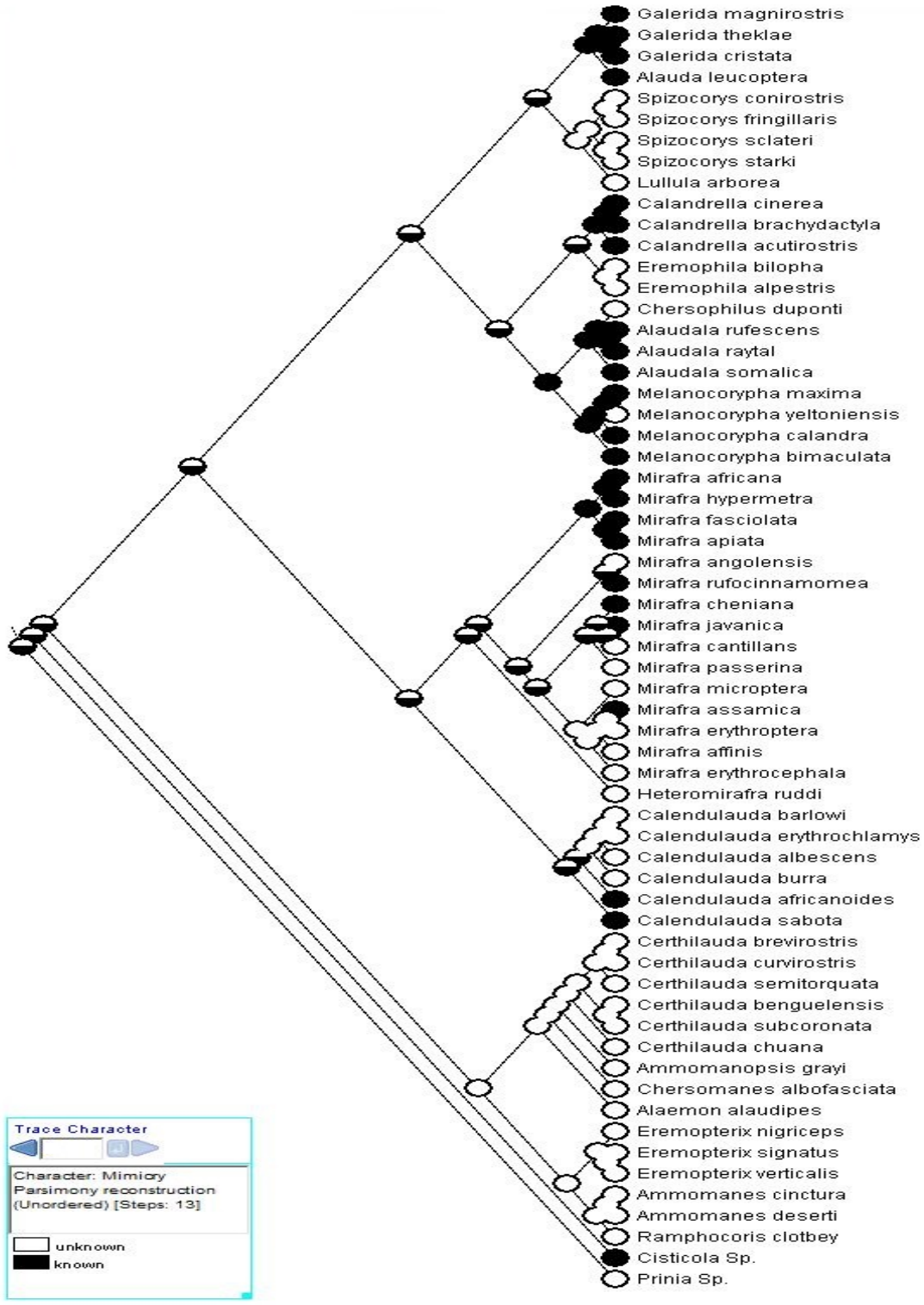


FIGURE 4.10. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF MIMICRY TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

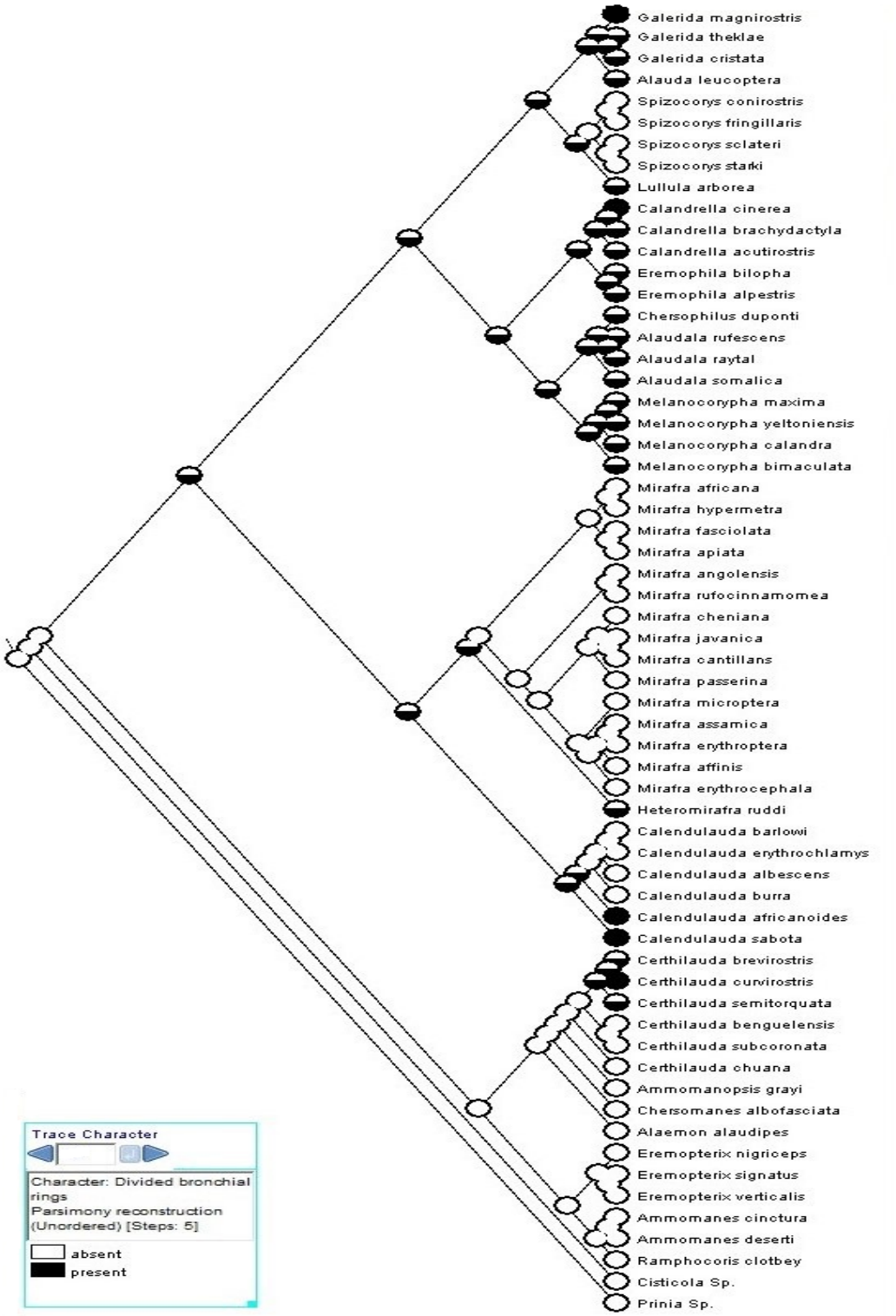


FIGURE 4.11. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF DIVIDED BRONCHIAL RINGS TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

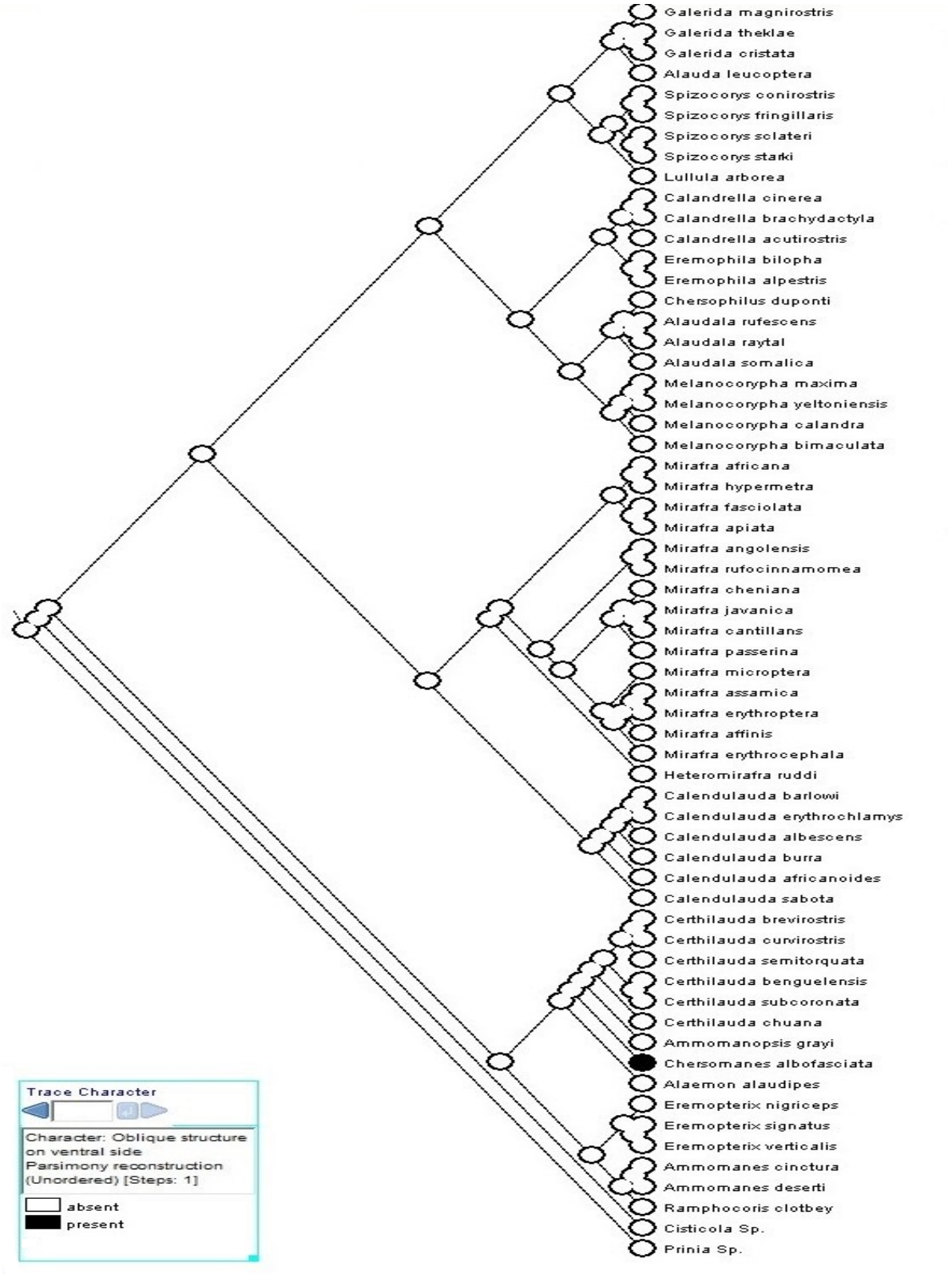


FIGURE 4.12. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF THE OBLIQUE STRUCTURE ON VENTRAL SIDE TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

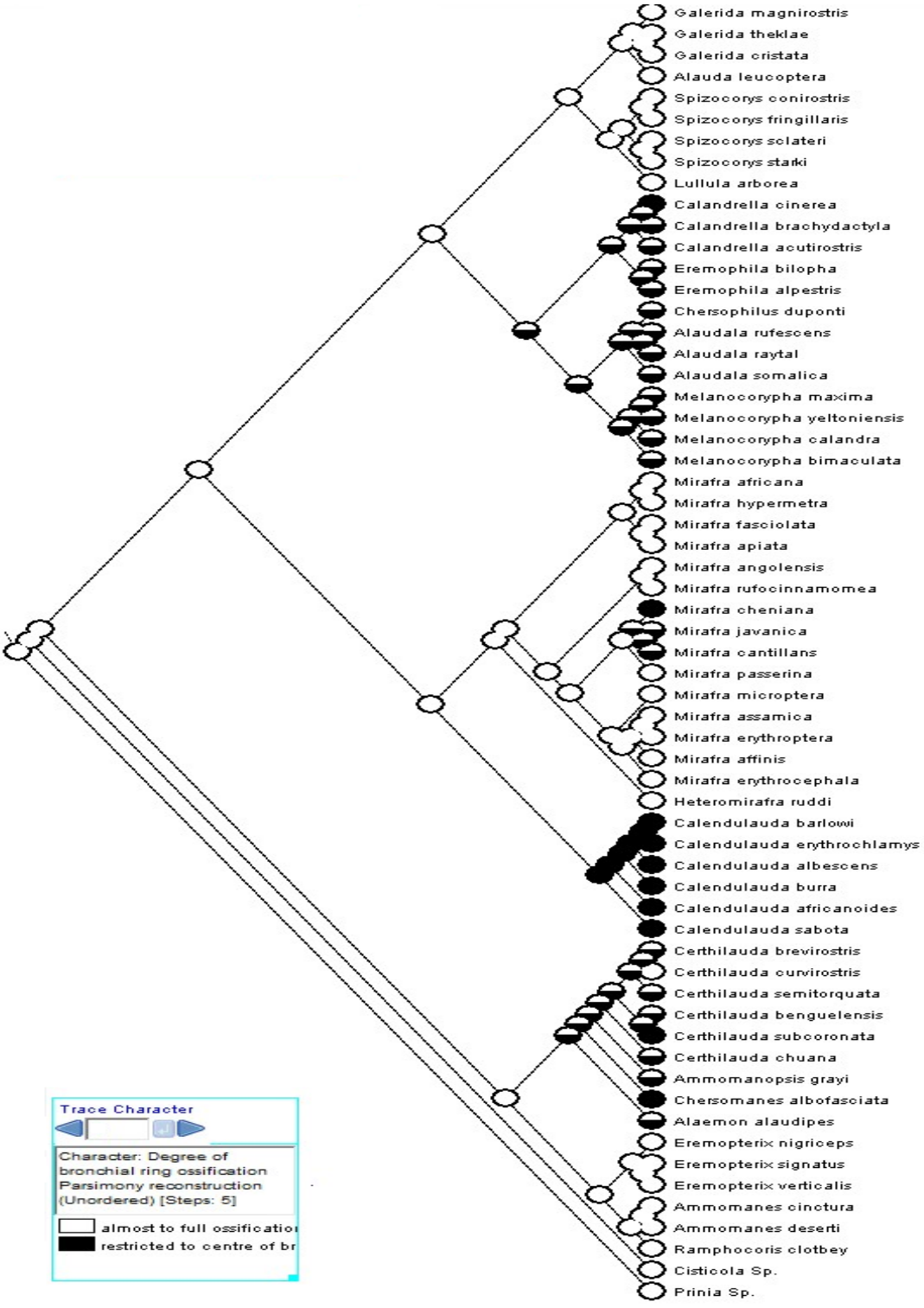


FIGURE 4 13. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF DEGREE OF BRONCHIAL RING OSSIFICATION TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

FIGURE 4.14. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF BRONCHIAL RING OSSIFICATION PATTERN TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

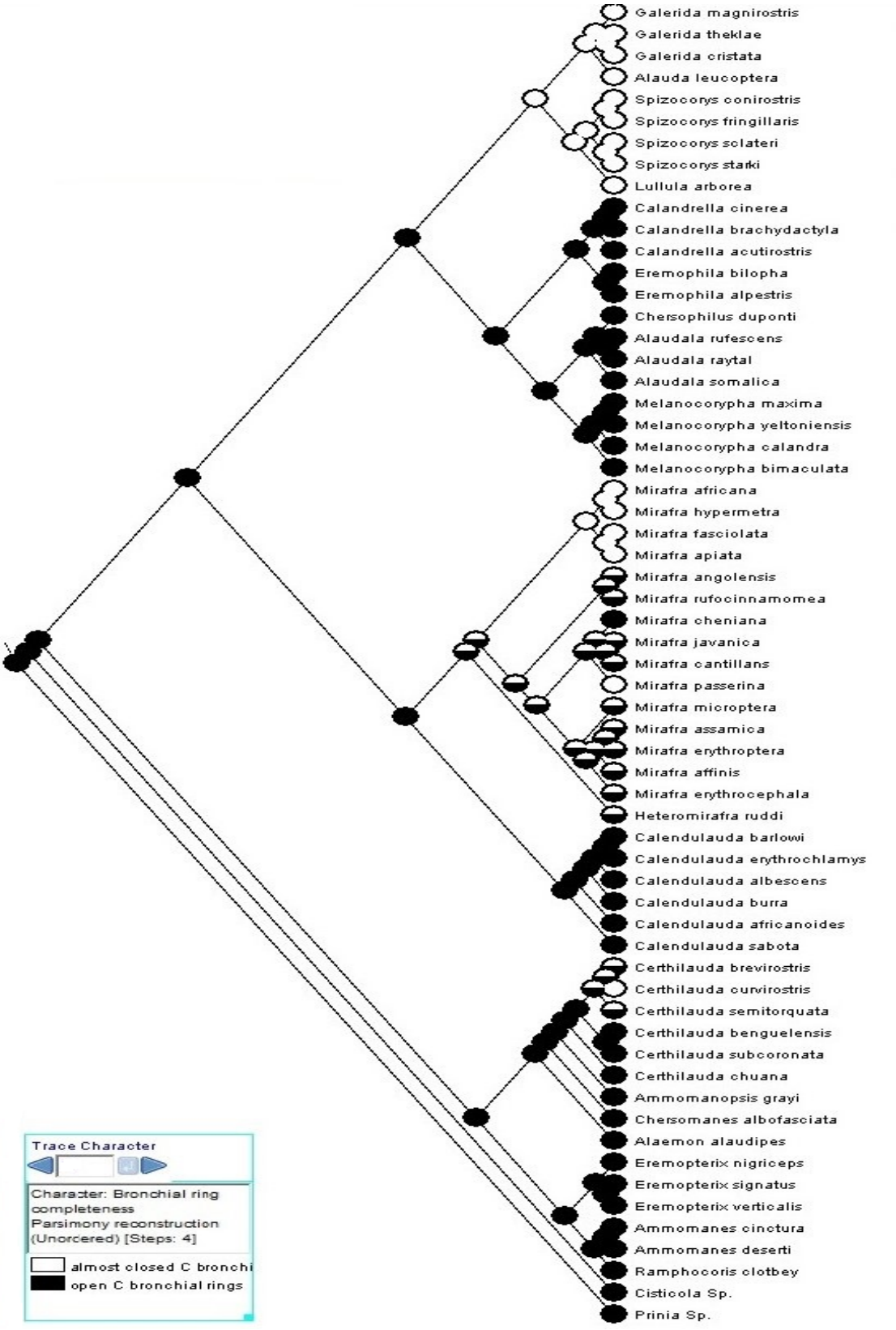


FIGURE 4.15. ANCESTRAL CHARACTER STATE RECONSTRUCTION BASED ON PARSIMONY OPTIMISATION OF BRONCHIAL RING COMPLETENESS TRACED ON A COMBINED MITOCHONDRIAL AND NUCLEAR DNA PHYLOGENY OF LARKS.

CHAPTER 5

Synthesis and future prospects

Upon commencement of the study, this research project was set out to address the following study questions:

i) can the structure of syringes and songs of larks be used to assess the distinctiveness of the three circumscribed major clades (A – Alaudid, B – Mirafrid, C – Ammomanid) in Alström *et al.* (2013)? [CHAPTER 2 & 3]
ii) how does the syringeal structure of the selected lark species compare in clade A (Alaudid), B (Mirafrid) and C (Ammomanid)? [CHAPTER 2]
iii) can songs be used to characterise the species of larks? [CHAPTER 3]
iv) how does the vocal phylogeny of larks compare to the molecular phylogeny? [CHAPTER 4]
v) how did the syringeal and song characters of larks evolve? [CHAPTER 4]

5.1 Highlights on key findings

Firstly, this study has demonstrated the importance of multiple sampling when studying the structure of the syrinx and vocalisations of larks. Multiple sampling in this context refers to whole syringes studied having been excised from different individual birds representing the same species. Similarly, multiple sampling also refers to the sampling of song strophes from songs rendered by different individual birds belong to the same species. Multiple sampling was key in order to reveal and learn about the syringeal and vocal variation that exist both intra- and interspecifically. Sometimes conclusions drawn based on a single specimen do not inspire confidence and from this, unfortunate conclusions may be reached.

The findings about the structure of the song strophes of the following three species is an example when multiple sampling affords researchers profound evidence: a Mirafrid (*Mirafra microptera*), an Ammomanid (*Certhilauda chuana*) and an Alaudid (*Alaudala raytal*) (see Chapter 3 for details). Multiple sampling allowed comparison with other taxa and helped in validating taxonomic decisions.

Another highlight from this study was on using the most unpopular evidence in the characterisation and to some degree the classification of larks. Using the syringeal and vocal characters to test the validity of the taxonomic circumscription of taxa is not fashionable. Molecular characters remain popular in systematics over the use of organismal characters and this is attributable to the benefits derived from the nature of characters (the nucleotides) when compared to the challenges presented by organismal characters. The number of potential characters available, rate independence between molecular and morphological evolution, and using molecular data in modelling patterns of nucleotide substitution are among some of the advantages of molecular characters. On the other hand, organismal characters are known to be susceptible to convergent evolution and most characters lacking a genetic basis. In this study, the difference was found pertaining to the phylogenetic structure of the larks between molecular and vocal phylogeny inferred through parsimony. Despite the same method of phylogenetic inference used, molecular phylogeny was consistent with what was found in Alström *et al.* (2013). The lark vocal phylogeny lacked a structure and this may be due to low number of characters used. The preference will be to have the number of characters not to be too far from the number of taxa studied. Another reason could be that the songs are highly variable among different song strophes from a single individual and across song strophes from different individual birds belong to the same species.

Gross morphological examination of the syringes showed very well that the syrinx of larks is loaded with large quantity of muscles especially on the ventral side that tend to hide a large part of the pessulus. This study examined the histology of the syrinx which then exposed the pessulus. The histological and gross morphological analyses complement each other even though the histological stain used does not have the ability to stain ossified or mineralised tissue components. The stain used for gross morphological examination included Alizarin red solution which stains bone components. Gross morphologically, ossified pessulus is slightly exposed (e.g. Fig. 2.2). Unfortunately, most of the specimens do not show this feature due to the bulk muscles surrounding the syrinx.

One of the key findings emerged when tracing the evolution of song and syringeal characters of larks. The main challenge is the fact that the outgroups of larks are uncertain. This has bearing in what happens in the states from the deeper to shallow

nodes. This is the reason the decision to choose the outgroups was carefully sought in literature. Reconstruction of ancestral states irrespective of whether they are conclusive or inconclusive cannot be interpreted with confidence when there is no certainty with the outgroups.

Despite the unpopular nature of using vocalisation and syringeal evidence in systematics, this study has demonstrated the potential that these characters have in studying the species of larks. The utility of vocalisation and syringeal characters comparatively has positives at different level of investigation than using for example, song characters to reconstruct phylogeny.

5.2 Future prospects

In science, citations give assurance that the information being presented has been proven previously to be true and based on published evidence. There is a difference between citations that can be traced back to the original source of information and those that can be traced back to what is considered to be the original source of information but with no information being located in a particular source. This may have been the reason that led to a large body of literature presenting different information about the pessulus in the lark syrinx. Pessulus has been deemed to be either lacking in the syrinx of larks, present but rudimentary or present but not bony. Our tracing back to the sources of this information did not help since no anatomical or gross morphological structures or any other relevant evidence could be located. It is recommended that the findings in research should be presented with full supporting evidence to avoid confusion. Undoubtedly, histology and gross morphological examination of syringes complement each other.

For future research, it will be critical to perform both gross morphological and histological analyses of the syringes of additional lark species to fulfil a good coverage and representation of the three clades (A – Alaudid, B – Mirafrid and C – Ammomanid). It will be important to use stains that have the ability to stain bone material, cartilage and also muscles. This will help in determining the level of development of the various tissue. Multiple sampling in terms of the different individual birds for vocalisation and syringeal consideration should be fulfilled to allow profound assessment of variation conspecifically. Further to this, birds should be sexed so that the effect of sex difference could be determined.

REFERENCES

Allsopp, N., Colville, J.F. and Verboom, G.A. 2014. *Ecology, Evolution and Conservation of a Megadiverse Region.* Oxford: Oxford University Press.

Alström, P. and Mild, K. 1993. The taxonomic status of *Anthus berthelotii. Bulletin of the British Ornithologists' Club* 113: 88-92.

Alström, P. and Ranft, R. 2003. The use of sounds in avian systematics and the importance of bird sound archives. *Bulletin of the British Ornithologists' Club* 123A: 114-135.

Alström, P. 1998. Taxonomy of the *Mirafra assamica* complex. *Forktail* 13: 97–107.

Alström, P. 2001. *The use of sounds in bird systematics. Introductory research essay no. 2.* Uppsala: Department of Systematic Zoology, Evolutionary Biology Centre, Uppsala University.

Alström, P., Barnes, K.N., Olsson, U., Barker, F.K., Bloomer, P., Khan, A.A., Qureshi, M.A., Guillaumet, A., Crochet, P.–A. and Ryan, P.G. 2013. Multilocus phylogeny of the avian family Alaudidae (larks) reveals complex morphology evolution, non–monophyletic genera and hidden species diversity. *Molecular Phylogenetics and Evolution* 69: 1043-1056.

Alström, P., Ericson, P.G.P., Olsson, U. and Sundberg, P. 2006. Phylogeny and classification of the avian superfamily Sylvioidea. *Molecular Phylogenetics and Evolution* 38: 381-397.

Alström, P., Olsson, U. and Lei, F. 2013. A review of the recent advances in the systematics of the avian superfamily Sylvioidea. *Chinese Birds* 4 (2): 99–131.

Alström, P., Olsson, U., Rasmussen, P.C., Yao, C.T., Ericson, P.G.P. and Sundberg, P. 2007. Morphological, vocal and genetic divergence in the *Cettia acanthizoides* complex (Aves: Cettiidae). *Zoological Journal of the Linnean Society* 149: 437-452.

Alves, M.J., Coelho, H., Collares–Pereira, M.J. and Coelho, M.M. 2001. Mitochondrial DNA variation in the highly endangered Cyrinid fish *Anaecypris hispanical*: Importance for conservation. *Hereditory (Edinburgh)* 87 (4): 463-473.

Amadon, D. 1950. The Hawaiian honeycreepers (Aves: Drepaniidae). *Bulletin of the American Museum of Natural History* 95 (4): 155–262.

Ames, P.L. 1971. The morphology of the syrinx in passerine birds. *Bulletin of the Peabody Museum of Natural History* 37: 1-241.

Appel, F.W. 1929. Sex dimorphism in the syrinx of the Fowl. *Journal of Morphology,* 47: 497–517.

Athreya, R. 2006. A new species of *Liocichla* (Aves: Timaliidae) from Eaglenest Wildlife Santuary, Arunachel Pradesh, India. *Indian Birds* 2: 82–94.

Bancroft J.D. and Gamble, M. 2002. *Theory and Practice of Histological Techniques.* 5th edition. New York: Churchill Livingstone.

Baptista, L.F. and Trail, P.W. 1992. The role of song in the evolution of passerine diversity. *Systematics Biology* 41: 242-247.

Barker, F.K., Barrowclough, G.F. and Groth, J.G. 2002. A Phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. *Proceedings of the Royal Society of London B* 269: 295-308.

Barnes, K.N. 2007. *The phylogenetics and evolution of Africa's Larks (Alaudidae).* PhD Thesis, University of Cape Town: Percy FitzPatrick Institute.

Bates, G.L. 1914. Some facts bearing on the affinities of *Smithornis*. *International Journal of Avian Science* 56: 495-502.

Baumel, J.J., King, A.S., Breazile, J.E, Evans, H.E. and Berge, J.C. 1993. *Handbook of Avian Anatomy: Nomina Anatomica Avium.* Vol. 23. Cambridge, MA: Nuttall Ornithological Club.

Beddard, F.E. and Parson, F.G. 1893. On certain points in the anatomy of parrots bearing their classification. *Proceedings of the Zoological Society of London* 506-514.

Bertelli, S. and Porzecanski, A.L. 2004. Tinamou (Tinamidae) Systematics: a preliminary combined analysis of morphology and molecules. *Ornitología Neotropical* 15 (Supplementary): 293-299.

Bertelli, S. and Tubaro, P.L. 2002. Body mass and habitat correlates of song structure in a primitive group of birds. *Biological Journal of the Linnean Society* 77: 423-430.

Bjarnason, A., Soligo, C. and Elton, S. 2015. Phylogeny, Ecology, and Morphological Evolution in the Atelid Cranium. *International Journal of Primatology* 36 (3): 513-529.

Blumstein, D. and Munos, O. 2005. Individual, age and sex–specific information is contained in yellow–bellied marmot alarm calls. *Animal Behaviour* 69: 353-361.

Blyth, E. 1938. Analytical descriptions of the groups of birds composing the order Strepitores. *The Annals and Magazine of Natural History* 2 (2): 589-601.

Bocek, M. and Bocak, L. 2017. The comparison of molecular and morphology–based phylogenies of *trichaline* net–winged beetles (Coleoptera: Lycidae: Metriorrhynchini) with description of a new subgenus. *PeerJ* 5: e3963.

Bock, W.J. 1972. Review of the morphology of the syrinx in passerine birds by P L Ames. *The Auk* 89: 899-903.

Boehmke, B. and Greenwell, B.M. 2019. *Hands-On Machine Learning with R.* 1st. Chapman & Hall/CRC The R Series.

Bonnevie, B.T. and Craig, A.J.F.K. 2018. A song for the South also: defining birdsong in global terms. *International Journal of Avian Science* 160 (4): 926-928.

Bookstein, F., Chernoff, B., Elder, R., Humphries, J., Smith, G. and Strauss, R. 1985. *Morphometrics in evolutionary biology: the geometry of size and shape change, with examples from fishes.* Philadelphia, PA: Academy of Natural Sciences of Philadelphia.

Bradbury, J.W. and Vehrencamp, S.L. 1998. *Principles of Animal Communication.* Sunderland, MA: Sinauer Associates.

Brown, C. and Ward, D. 1990. The morphology of the syrinx in the Charadriiformes (Aves): possible phylogenetic implications. *Bonner Zoologische Beiträge* 41: 95-107.

Cannell, P.F. 1988. Techniques for study of avian syringes. *The Auk* 100: 289-293.

Cap, H., Deleporte, P., Joachim, J. and Reby, D. 2008. Male vocal behavior and phylogeny in deer. *Cladistics* 24: 917-931.

Carneiro, L.S. and Aleixo, A. 2014. Molecular phylogenetics and chronology of the genus Hylopezus (Grallariidae). *Cuzco: Abstracts of the IX Congresso de Ornitología Neotropical y el VIII Congreso Peruano de Ornitología.*

Catchpole, C.K. and Slater, P.J.B. 2008. *Bird song: Biological Themes and Variations.* 2nd. Cambridge: Cambridge University Press.

Catchpole, C.K. and Slater, P.J.B. 1995. *Bird song: Biological Themes and Variations.* Cambridge: Cambridge University Press.

Catchpole, C.K. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour,* 74: 149-166.

Chamberlain, D. R., Gross, W.B., Cornwell, G.W. and Mosby, H.S. 1968. Syringeal anatomy of the common crow. *The Auk* 85 (2): 244-252.

Chen, Z. and Maher, R.C. 2006. Semi–automatic classification of bird vocalizations using spectral peak tracks. *Journal of the Acoustical Society of America* 120 (5): 2374-2984.

Christensen, L.A., Allred, L.M. Goller, F. and Meyers, R.A. 2017. Is sexual dimorphism in singing behavior related to syringeal muscle composition? *The Auk* 134 (3): 710-720.

Christensen, L.A., Meyers, R.A. and Goller F. 2014. Lack of song in females does not drive sexual dimorphism in syringeal muscle composition. *Integrative and Comparative Biology* 54 (Supplement): e253.

Clancey, P.A. 1980. *S.A.O.S. Checklist of Southern African Birds.* Johannesburg: Southern African Ornithological Society.

Clancey, P.A. 1966. Subspeciation in southern African populations of Sabota Lark *Mirafra sabota* Smith. *Ostrich: Journal of African Ornithology* 37: 207-213.

Clark, H.L. 1913. Anatomical notes on *Todus*, *Oxyruncus* and *Spindalis*. *The Auk* 30: 402-405.

Collins, M.D., Lawson, P.A., Willems, A., Cordoba, J.J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. and Farrow, J.A.E. 1994. The phylogeny of the genus *Clostridium*: proposal five new genera and eleven new species combinations. *International Journal of Systematic Bacteriology* 44: 812-82.

Cracraft, J., Barker, F.K., Braun, M., Harshman, J., Dyke, G.J., Feinstein, J., Stanley, S., Cibois, A., Schikler, P., Beresford, P., García–Moreno, J., Sorenson, M.P., Yuri, T. and Mindell, D.P. 2004. Phylogenetic relationships among modern birds (Neornithes): toward an avian tree of life. In *Assembling the Tree of Life*, by Cracraft, J. and Donoghue, M.J. (eds.). 468–489. Oxford: Oxford University Press.

Cruickshank, A., Gautier, J. and Chappuis, C. 1993. Vocal mimicry in wild African Grey Parots *Psittacus arithacus*. *International Journal of Avian Science* 135 (3): 293–299.

Cuvier, G.L.F.D. 1802. Ueber den untern Larynx der Vogel. *Archiv fur die Physiologie (Halle)* 5: 67-96.

De Beer, S.J., Lockwood, G.M., Raijmakers, J.H.F.A., Raijmakers, J.M.H., Scott, W.A., Oschadleus, H.D. and Underhill, L.G. 2001. *SAFRING Bird Ringing Manual.* ADU Guide 5. Cape Town: Avian Demography Unit.

de Juana, E., Suárez F., Ryan P., Alström P. and Donald P. 2004. *Family Alaudidae (Larks).* Vol. 9, in *Handbook of the Birds of the World*, by del Hoyo, J., Elliott, A. and Christie, D.A. (eds.). 496-601. Barcelona: Lynx Edicions.

de Juana, E., Suárez, F. and Ryan, P. 2020. *Larks (Alaudidae).* del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. and de Juana, del Hoyo, E. (eds.). Barcelona: Lynx Edicions. Accessed May 8, 2020. http://www.hbw.com/node/52302.

de Juana, E. and Suárez, F. 2020. Dupont's Lark (*Chersophilus duponti*), v. 1.0, in *Birds of the World*, by Billerman, S.M., Keeney, B.K., Rodewald, P.G. and Schulenberg, T.S. (eds.). New York, Ithaca, Cornell Lab of Ornithology.

Dean, W.R.J. and Hockey, P.A.R. 1989. An ecological perspective of lark (Alaudidae) distribution and diversity in the south–west arid zone of Africa. *Ostrich: Journal of African Ornithology* 60: 27-34.

Dean, W.R.J., Fry, C.H., Keith, S. and Lack, P. 1992. Family Alaudidae: larks. In *The Birds of Africa*, by Keith, S., Urban, E.K. and Fry, C.H. (eds.). 13–124. London: Academic Press.

Dean, W.RJ. and Williams, J.B. 2004. Adaptations of birds for life in the deserts with particular reference to the larks (Alaudidae). *Transactions of the Royal Society of South Africa* 59: 79-90.

del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. and de Juana, E. 2019. *In Birds of the World.* Ithaca, NY: Cornell Lab of Ornithology.

Delacour, J. and Mayr, E. 1945. The family Anatidae. *Wilson Bulletin* 57: 3-55.

Delhey, K. 2019. A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. Biological Reviews 94 (4): 1294-1316.

Derryberry, E.P., Seddon, N., Claramunt, S., Tobias, J.A., Baker, A., Aleixo, A. and Brumfield, R.T. 2012. Correlated evolution of beak morphology and song in the neotropical woodcreeper radiation. *Evolution* 66: 2784-2797.

Desutter–Grandcolas, L. and Robillard, T. 2004. Phylogeny and the evolution of calling songs in *Gryllus* (Insecta, Orthoptera, Gryllidae. *Zoologica Scripta* 32: 173-183.

Dickinson, E. 2003. *The Howard and Moore Complete Checklist of the Birds of the World.* New Jersey: Princeton University Press.

Donald, P.F. 2004. *The Skylark.* London: T and A.D. Poyser Publishers.

Donald, P., Alström, P., & Engelbrecht, D. 2017. Possible mechanisms of substrate colour-matching in larks (Alaudidae) and their taxonomic implications. *Ibis* 159: 699-702.

Düring, D.N., Ziegler, A., Thompson, C.K., Ziegler, A., Faber, C., Müller, J., Scharff, C., Elemans, C.P.H. 2013. The songbird syrinx morphome: A three-dimensional, high–resolution, interactive morphological map of the zebra finch vocal organ. *BMC Biology* 11: 1-27.

Dyce, K.M., Sack, W.O. and Wensing, C.J.G. 1996. *Textbook of Veterinary Anatomy.* 2nd. Philadelphia: W.B. Saunders Co.

Edwards, S.V., Kingan, S.B., Calkins, J.D., Balakrishnan, C.N., Jennings, W.B., Swanson, W.J. and Sorenson, M.D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proceedings of the National Academy of Sciences of the United States of America* 102: 6550-6557.

Elemans, C.P.H., Muller, M., Larsen, O.N and van Leeuwen, J.L. 2009. Amplitude and frequency modulation control of sound production in a mechanical model of the avian syrinx. *Journal of Experimental Biology* 212: 1212-1224.

Engelbrecht, D. and Dikgale M.L. 2017. Display and vocalizations of the Chestnut-backed Sparrowlark. *Honeyguide* 63: 35-42.

Ericson, P.G.P., Zuccon, D., Ohlson, J.I., Johansson, U.S., Alvarenga, H. and Prum, R.O. 2006. Higher–level phylogeny and morphological evolution of tyrant flycatchers, cotingas, manakins, and their allies (Aves: Tyrannida). *Molecular Phylogenetics and Evolution* 40: 471-483.

Eyton, T.C. 1841. Notes on birds. No 2. *The Annals and magazine of natural history* 7: 177-179.

Falls, J.B. 1982. Individual recognition by sound in birds.” In *Acoustic communication in birds*, by Kroodsma, D.E. and Miller, E.H. (eds.). 237-273. New York: Academic Press.

Farnsworth, A. and Lovette, I.J. 2008. Phylogenetic and ecological effects on interspecific variation in structurally simple avian vocalizations. *Biological Journal of the Linnean Society* 95: 155-173.

Felsenstein, J. 1984. Distance methods for inferring phylogenies: a justification. *Evolution* 38 (1): 16-24.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootsrap. *Evolution* 39 (2): 783-791.

Fischer, M. and Thatte, B. 2010. Revisiting an Equivalence Between Maximum Parsimony and Maximum Likelihood Methods in Phylogenetics. *Bulletin of Mathematical Biology* 72: 208-220.

Fleagle, J.G. 2013. *Primate adaptation and evolution.* 2nd edition. San Diego: Academic Press.

Fletcher, N.H. and Tarnopolsky, A. 1999. Acoustics of the avian vocal tract. *Journal of Acoustical Society of America* 105: 35–49.

Forbes, W.A. 1880a. Contributions to the anatomy of passerine birds. Part 2. On the syrinx and other points in the anatomy of the Eurylaemidae. *Proceedings of the Zoological Society of London* 1880: 380–386.

Forbes, W.A. 1880b. Contributions to the anatomy of passerine birds. Part 3. On some points in the structure of *Philepitta* and its position amongst the Passeres. *Proceedings of the Zoological Society of London* 1880: 387–391.

Forbes, W.A. 1881. Contributions to the anatomy of passerine birds. Part 4. On some points in the anatomy of the genus *Conopophaga* and its systematic position. *Proceedings of the Zoological Society of London* 1881: 435–438.

Forbes, W.A. 1882. Contributions to the anatomy of passerine birds. Part 6. On *Xenicus* and *Acanthisitta* as types of a new family (Xenicidae) of Mesomyodean passeres from New Zealand. *Proceedings of the Zoological Society of London* 1882: 569–571.

Frank, T., Walter, I., Probst, A., and König, H.E. 2007. Histological aspects of the syrinx of the mallard (*Anas platyrhynchos*). *Anatomia, Histologia and Embryologia* 36: 121–126.

Fregin, S., Haase, M., Olsson, U. and Alström, P. 2012. New insights into family relationships within avian superfamily Sylvioidea (Passeriformes) based on seven molecular markers. *BMC Evolutionary Biology* 12: 157.

Fürbringer, M. 1888. *Untersuchungen zur Morphologia und Systematik der Vogel, zugleich ein Beitrag zur Anatomie der Stutz– und Bewegungsorgane. II. Allegemeiner Tei.* Amsterdam: Smithsonian Libraries.

Gaban–Lima, R. and Höfling, E. 2006. Comparative anatomy of the syrinx in the tribe Arini (Aves: Psittacidae). *Brazilian Journal of Morphological Sciences* 23: 501–512.

Gadow, H. and Selenka, E. 1893. *Klassen und Ordenungen des Thier–Reichs. Sechzeter Band*. In *Vogel. Vol. 2, Anatomischer Teil*, by Bronn's, G.F. and Winter, L., 1008. Viert Abhandl.

García, N.C., Barreira, A.S., Kopuchian, C. and Tubaro, P.L. 2014. Intraspecific and interspecific vocal variation in three Neotropical cardinalids (Passeriformes: Fringillidae) and its relationship with body mass. *Emu – Austral Ornithology* 114: 129–136.

Garcia, S.M., Kopuchian, C., Mindlin, G.B., Fuxjager, M.J., Tubaro, P.L and Goller, F. 2017. Evolution of vocal diversity through morphological adaptation without vocal learning or complex neural control. *Current Biology* 27 (17): 2677–2683.

Garrod, A.H. 1876. On some anatomical peculiarities which bear upon the major divisions of passerine birds. *The Proceedings of the Zoological Society of London* 506–519.

Garrod, A.H. 1877. Notes on the anatomy of passerine birds. *The Proceedings of the Zoological Society of London* 447–453.

Gaunt A.S. and Gaunt S.L. 1985. *Syringeal structure and avian phonation.* Vol. 2, in *Current Ornithology*, by Johnston R.F. (ed.). 213–245. New York: Plenum Press.

Gaunt, A.S. and Gaunt, S.L. 1977. Mechanics of the syrinx in *Gallus gallus*. *Journal of Morphology* 152: 1–20.

Gill, F.B. and Donsker, D. 2012. *IOC World Bird List (v. 3.1). [Online].* 25 June http:// www.worldbirdnames.org.

Gill, F.B. and Donsker, D. 2020. *IOC World Bird List (v. 7.3). [Online].* 17 May. http://worldbirdsnames.org.

Gill, F.B. 1990. *Ornithology.* New York: Freeman, W.H. and Company.

Gill, F.B. 2007. *Ornithology: Behavior and communication.* 3rd. New York: Freeman, W.H. and Company.

Goldberg, T.L. 2003. Application of phylogeny reconstruction and character–evolution analysis to inferring patterns of directional microbial transmission. *Preventive Veterinary Medicine* 61: 59–70.

Goldwave Inc. 2015. *Goldwave (Version 6.15).* Computer software, www.goldwave.com

Greenewalt, G.H. 1968. *Birdsong: acoustics and physiology.* Washington: Smithsonian Institution Press.

Griffiths, C.S. 1994a. Monophyly of the Falconiformes based on syringeal morphology. *The Auk* 111: 787–805.

Griffiths, C.S. 1994b. Syringeal morphology and the phylogeny of the Falconidae. *Condor* 96: 127–140.

Guillaumet, A., Crochet, P.A. and Godelle, B. 2005. Phenotypic variation in *Galerida* larks in Morocco: the role of history and natural selection. *Molecular Ecology* 14: 3809–3821.

Guillaumet, A., Crochet, P.A. and Pons, J.–M. 2008. Climate–driven diversification in two widespread *Galerida* larks. *BMC Evolutionary Biology* 8: 32.

Haecker, V. 1900. Der Gesang der Vogel, seine anatomischen und biologischen Grundlagen. *Gustav Fischer, Jena* 102.

Hall, B.K. 2007. Homoplasy and homology: Dichotomy or continuum? *Journal of Human Evolution* 52: 473–479.

Hammer, Ø., Harper, D.A.T. and Ryan, P.D. 2005. PAST: Paleontological Statistics Software package for education and data analysis. *Paleontological Electronica* 4 (1): 9.

Harrington, R.C., Benavides, E. and Near, T.J. 2012. Phylogenetic inference of nuptial trait evolution in the context of phylogenetic inference of nuptial trait evolution in the context of asymmetrical introgression in North American darters (Teleostei). *Evolution* 67 (2): 388–402.

Harrison, C.J.O. 1966. The validity of some genera of larks (Alaudidae). *International Journal of Avian Science* 108: 573–583.

Hauser, M.D. 1993. The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *The American Naturalist* 142: 528–542.

Helm, S. Herissant, H. 1753. Récherches sur les organes de la voix des quadrupedes et de celle des oiseaux, Academie des Sciences Paris. *Mémoires* 179–194.

Herre, R. 1859. *De avium passerinarum larynge bronchiali.* Gryphiae: Dissertation anatomica.

Hillis, D.M. and Wiens, J.J. 2000. Molecules versus morphology in systematics: Conflicts, artifacts, and misconceptions. In *Phylogenetic analysis of morphological data*, by Wiens, J.J. (ed.). 1–19. Washington DC: Smithsonian Institution Press.

Ho, S.Y.W. and Jermiin L.S. 2004. Tracing the decay of the historical signal in biological sequence. *Systematic Biology* 53: 623–637.

Hockey, P.A.R., Dean, W.R.J. and Ryan, P.G. 2005. *Roberts–Birds of Southern Africa.* 7th. Cape Town: The trustees of John Voelcker Bird Book Fund.

Huelsenbeck, J.P. and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Applications Note* 17 (8): 754–755.

Huelsenbeck, J.P., Nielsen, R. and Bollback, J.P. 2003. Stochastic mapping of morphological characters. *Systematic Biology* 52 (2): 131–158.

Humphrey, P.S. 1955. *The relationships of the seaducks (tribe Mergini).* PhD. Thesis, Ann Arbor: University of Michigan.

Huxley, T.H. 1867. On the classification of birds; and on the taxonomic value of the modification of certain of the cranial bones observable in that class. *Zoological Society of London* 415–472.

I**báñez, A., Lòpez, P. and Martín, J.** 2014. Inter–individual variation in antipredator hiding behaviour of Spanish terrapins depends on sex, size, and coloration. *Ethology* 120: 742–752.

Ilves, K.L. and Taylor, E.B. 2009. Molecular resolution of the systematics of a problematic group of fishes (Teleostei: Osmeridae) and evidence for morphological homoplasy. *Molecular Phylogenetics and Evolution* 50: 163–178.

Irestedt, M., Fjeldså, J., Johansson, U.S. and Ericson, P.G.P. 2002. Systematic relationships and biogeography of the tracheophone Suboscines (Aves: Passeriformes). *Molecular Phylogenetics and Evolution* 23 (3): 499–512.

Irwin, D.E. 2000. Song variation in an avian ring species. *Evolution* 54: 998–1010.

I**sler, M.L., Bravo, G.A. and Brumfield, R.T.** 2013. Taxonomic revision of *Myrmeciza* (Aves: Passeriformes: Thamnophilidae) into 12 genera based on phylogenetic, morphological, behavioral, and ecological data. *Zootaxa* 3717 (4): 469–497.

Isler, M.L., Isler, P.R. and Whitney, B.M. 1998. Use of vocalizations to establish species limits in antbirds (Passeriformes: Thamnophilidae). *The Auk* 577–590.

Iwaniuk, A.N., Hurd, L. and Wylie, D.R.W. 2006. The Comparative Morphology of the Cerebellum in Caprimulgiform Birds: Evolutionary and Functional Implications. *Brain Behavior and Evolution* 67: 53–68.

Jarvis, E.D. 2006. Evolution of brain structures for vocal learning in birds: a synopsis. *Acta Zoologica Sinica* 52: 985–89.

Jenner, R.A., Dhubhghaill, C.N., Ferla, M.P. and Wills, M.A. 2009. Eumalacostracan phylogeny and total evidence: Limitations of the usual suspects. *BMC Evolutionary Biology* 9: 21.

Johnsgard, P.A. 1961. Tracheal anatomy of the Anatidae and its taxonomies significance. *Wildfowl* 12: 58–69.

Jollife, I.T. 2002. *Principal Component Analysis.* 2nd. New York: Springer–Verlag.

Joy, J.B., Liang, R.H., McCloskey, R.M., Nguyen, T. and Poon, A.F.Y. 2016. Ancestral Reconstruction. *PLoS Computer Biology* 12 (7): e10004763.

Karanovic, T., Kim, K. and Lee. W. 2015. Concordance between molecular and morphology–based phylogenies of Korean *Enhydrosoma* (Copepoda: Harpacticoida: Cletodidae) highlights important synapomorphies and homoplasies in this genus globally. *Zootaxa* 3990 (4): 451–596.

Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron-microscopy. *Journal of Cell Biology* 27: 137–138A.

Keith, S., Urban, E.K. and Fry, C.H. 1992. *The Birds of Africa.* Vol. 4. London: Academic Press.

Kelley, L.A., Coe, R.L., Madden, J.R. and Healy, S.D. 2008. Vocal mimicry in songbirds. *Animal Behaviour* 76: 521–528.

King, A. S., and McLelland, J. 1984. *Birds: their Structure and Function.* 2nd edition. London: Bailliere and Tindall.

King, A.S. 1989. Functional anatomy of the syrinx. In *Form and function in birds*, by King, A. and McLelland, J. (eds.). 105–192. New York: Academic Press.

Köditz, W. 1925. Ueber die Syrinx einiger Clamatores und auslandischer Oscines, Zeitschr. fiir Wissensch. *Zoologia* 126 (1): 70–144.

Krabbe, N. and Cadena, C.D. 2010. A taxonomic revision of the Paramo Tapaculo *Scytalopus canus* Chapman (Aves: Rhinocryptidae), with description of a new subspecies from Ecuador and Peru. *Zootaxa* 2354: 56–66.

Krakauer, A.H., Tyrrell, M., Lehmann, K., Losin, N., Goller, F. and Patricelli, G.L. 2009. Vocal and anatomical evidence for two–voiced sound production in the greater sage–grouse *Centrocercus urophasianus*. *Journal of Experimental Biology* 212: 3719–3727.

Kroodsma, D.E. and Candy, R.A. 1985. Differences in repertoire size, singing behavior, and associated neuroanatomy among marsh wren populations have a genetic basis. *The Auk* 102: 439–446.

Kroodsma, D.E. and Konishi, M. 1991. A Suboscine bird (eastern phobe, *Sayornis phoebe*) develops normal song without auditory feedback. *Animal Behaviour* 42: 477–487.

Kroodsma, D.E. 1977. A re–evaluation of song development in the Song Sparrow. *Animal Behaviour* 25: 390–399.

Kroodsma, D.E. 1981. Geographic variation and functions of song types in warblers (Parulidae). *The Auk* 98: 743–751.

Kroodsma, D.E. 1982. Learning and the ontogeny of sound signals in birds. In *Acoustic communication in birds*, by Kroodsma, D.E. and Miller, E.H. (eds.). 1–23. New York: Academic Press.

Kroodsma, D.E. 1984. Songs of the Alder Flycatcher (*Empidonax alnorum*) and Willow Flycatcher (*Empidonax traillii*) are innate. *The Auk* 101: 13–24.

Kuchler, W. 1936. Anatomisch Untersuchungen an Phytotoma rara. *Journal für Ornithologie* 84: 352–362.

Kułaga, K. and Budka, M. 2019. Bird species detection by an observer and an autonomous sound recorder in two different environments: Forest and farmland. *PLoS ONE* 14 (2): 1.19.

Kuroda, N. 1990. *The Jungle Crows of Tokyo.* Tokyo: Yamashina Institute for Ornithology.

Lack, D. 1947. *Darwin's Finches.* Cambridge: Cambridge University Press.

Lanyon, S.M. and Lanyon, W.E. 1989. The systematic position of the plantcutters, *phytotoma*. *The Auk* 106: 422–432.

Lanyon, W.E. 1969. Vocal characters and avian systematics. In *Bird vocalizations: their relation to current problems in Biology and Psychology*, by Hinde, R.A. (ed). 291–310. London: Cambridge University Press.

Lanyon, W.E. 1979. Development of song in the Wood Thrush (*Hylocichla mustelina*), with notes on a technique for Passerines from the egg. *American Museum Novitates* 2666: 1–27.

Lanyon, W.E. 1986. A phylogeny of the thirty–three genera in the *Empidonax* assemblage of tyrant flycatchers. *American Museum Novitates* 2846: 1–64.

Larsen, O.N. and Goller, F. 2002. Direct observation of syringeal muscle function in songbirds and a parrot. *Journal of Experimental Biology* 205: 25–35.

Lei, F., Wang, A., Wang, G. and Yin, Z. 2005. Vocalisations of Red–necked snow Finch, *Pygilauda ruficollis* on the Tibetan Plateau, China – a syllable taxonomic signal? *Folia Zoologica* 54 (1–2): 135–146.

Lein, M.R. 1978. Song variation in a population of Chestnut–sided Warblers (*Dendroica pensylvanica*): Its nature and suggested significance. *Canadian Journal of Zoology* 56: 1266–1283.

Leisler, S., Heidrich, P., Schulze–Hagen, K. and Wink, M. 1997. Taxonomy and phylogeny of reed warblers (genus *Acrocephalus*) based on mtDNA sequences and morphology. *Journal of Ornithology* 138: 469–496.

Livezey, B.C. 1986. A phylogenetical analysis of recent *Anseriform* genera using morphological characters. *The Auk* 103: 737–754.

Lloyd, P. 1999. Rainfall as a breeding stimulus and clutch size determinant in South African arid–zone birds. *International Journal of Avian Science* 141: 637–643.

Lowe, P.R. 1931. On the anatomy of *Pseudocalyptomena* and the occurrence of broadbills (Eurylaemidae) in Africa. *Proceedings of the Zoological Society of London* 100: 445–461.

Lowe, P.R. 1924. On the presence of broadbills (Eurylaemidae) in Africa. *Proceedings of the Zoological Society of London* 1924: 279–291.

Lowe, P.R. 1942. The anatomy of Gould's manakin (*Manacus vitellinus*) in relation to its display, with special reference to an undescribed pterylar tract and the attachments

of the Flexor carpi ulnaris and Flexor sublimis digitorum muscles to the secondary feathers. *International Journal of Avian Science* 83: 50–83.

Luine, V., Nottebohm, F., Harding, C. and McEwen, B.C. 1980. Androgen affects cholinergie enzymes in syringeal motor neurons and muscle. *Brain Research* 192: 89–107.

Macdonald, J.D. 1952a. Notes on the Long–bill Lark *Certhilauda curvirostris*. *International Journal of Avian Science* 94: 122–127.

Macdonald, J.D. 1952b. Notes on the taxonomy of the clapper larks of South Africa. *International Journal of Avian Science* 94: 629–635.

Macdonald, J.D. 1953. Taxonomy of the Karoo and Red–back Larks of western South Africa. *Bulletin of the British Museum of Natural History Zoology* 1: 319–350.

MacGillivray, W. 1839. *History of British birds.* 2nd. London: Scott Webster and Geary.

Maclean, G.L. 1969. South African Lark genera. *Cimbebasia* 1 (Series A): 79–94.

Maclean, G.L. 1970. The biology of the larks (Alaudidae) of the Kalahari sandveld. *Zoologia Africana* 5: 7–39.

Maddison, W.P. and Maddison, D.R. 2000. *MacClade 4: Analysis of Phylogeny and Character Evolution.* Version. 4.0., Sunderland, MA: Sinauer Associates.

Maddison, W.P. and Maddison, D.R. 2017. *Mesquite: a modular system for evolutionary analysis Version. 1.01.* http: //mesquiteproject.org. Google Scholar.

Maddisson, D.R. 1990. *Phylogenetic inference of historical pathways and models of evolution change.* PhD Thesis, Cambridge, MA: Harvard University.

Mandiwana–Neudani, T.G., Bowie, R.C.K., Hauberger, M., Henry, L. and Crowe, T.M. 2014. Taxonomic and phylogenetic utility of variation in advertising calls of *francolins* and *spurfowls* (Galliformes: Phasianidae). *African Zoology* 49 (1): 54–82.

Mandiwana–Neudani, T.G., Kopuchian, C., Louw, G. and Crowe, T.M. 2011. A study of gross morphological and histological syringeal features of true *francolins* (Galliformes: *Francolinus, Scleroptila, Peliperdix* and *Dendroperdix* spp.) and

spurfowls (*Pternistis* spp.) in a phylogenetic context. *Ostrich: Journal of African Ornithology* 82: 115–126.

Marler, P. 1955. Characteristics of some animal calls. *Nature* 176: 6–7.

Mashigo, T. 2018. *Geographical variation and characterisation of the vocalisation of the Short–clawed Lark Certilauda chuana.* Honours Mini–dissertation, University of Limpopo.

Mayr, E. and Amadon, D. 1951. A classification of Recent birds. *American Museum Novitates* 1496.

Mayr, E. 1931. Die Syrinx einiger Singvogel aus Neu Guinea. *Journal für Ornithologie* 79: 333–337.

McCracken, K.G. and Sheldon, F.H. 1997. Avian vocalizations and phylogenetic signal. *Proceedings of the National Academy of Sciences of the United States of America* 94: 3833–3836.**Meinertzhagen, R.** 1951. Review of the Alaudidae. *Proceedings of the Zoological Society of London* 121 (1): 81–132.

Mekonen, A. 2017. Birds as biodiversity and environmental indicator. *Journal of Natural Sciences Research* 7 (21): 28–34.

Mennill, D.J., Doucet, S.M., Newman, A.E.M., Williams, H., Moran, I.G., Thomas, I.P., Woodworth, B.K. and Norris, D.R. 2018. Wild Birds Learn Songs from Experimental Vocal Wild Birds Learn Songs. *Current Biology* 28: 1–6.

Miller, E.H. and Baker, A.J. 2009. Antiquity of shorebird acoustic displays. *The Auk* 122: 6–11.

Miskimen, M. 1951. Sound production in passerine birds. *The Auk* 68: 493–504.

Miskimen, M. 1963. The syrinx in certain tyrant flycatchers. *The Auk* 80: 156–165.

Mobley, J.A. and Prum, R.O. 1995. Phylogenetic relationships of the Cinnamon tyrant, *Neopipo cinnamomea*, to the Tyrant flycatchers (Tyrrannidae). *The Condor* 97: 650–662.

Moreau, R.E. 1966. *The Bird Faunas of Africa and its Islands.* London: Academic Press.

Mortimer, J.A.J., Greene, T.C. and Mortimer, S.P. 2018. Assessing bird vocalisation identification accuracy using a computer–based quiz. *New Zealand Journal of Zoology* 46 (3): 201–224.

Morton, E.S. 1975. Ecological sources of selection on avian sounds. *The American Naturalist* 109: 17–34.

Müller, J. 1847. *Ueber die bisher unbekannten Typischen Verschiedenheiten der Stimmorgane der Passerinen.* Konnig. Akad. Wiss., Berli: Abhandl.

Müller, J. 1878. *On certain variations in the vocal organs of the Passeres that have hitherto escaped notice (Translation by J. Bell of Müller 1847).* London: The Ray Society.

Myers, J.A. 1917. Studies on the syrinx of *Gallus domesticus*. *Journal of Morphology* 29: 1652–1714.

Naguib, M. and Price, J.J. 2013. The evolution of animal communication. *Behaviour* 150: 951–955.

Navarro–sigüenza, A.G. and Peterson, A.T. 2004. An alternative species taxonomy of the birds of mexico. *Biota Neotropica* 4 (2): 1–32.

Nicholls, J.A. and Goldizen, A.W. 2006. Habitat type and density influence vocal signal design in satin bowerbirds. *Journal of Animal Ecology* 75: 549–558.

Nielsen, R. 2002. Mapping mutations on phylogenies. *Systematic Biology* 51 (5): 729–739.

Nitzsch, G.L. 1829. Observationes de Avium Arteria carotide communi. *Halle* 26.

Ortega, J., Lòpez, P. and Martín, J. 2014. Conspicuous blue tails, dorsal pattern morphs and escape behaviour in hatchling Iberian wall lizards (*Pordarcis hispanicus*). *Biological Journal of the Linnean Society* 113: 1094–1106.

Owen, R. 1866. *Anatomy of vertebrates.* Vol. 2. London: Longmans, Green and Company.

Päckert, M., Martens, J., Kosuch, J., Nazarenko, A.A. and Veith, M. 2003. Phylogenetic signal in the song of crests and kinglets (Aves: *Regulus*). *Evolution* 57: 616–629.

Päckert, M., Martens, J., Sun, Y. and Veith, M. 2004. The radiation of the Seicercus burkii complex and its congeners (Aves: Sylviidae): molecular genetics and bioacoustics. *Organisms Diversity and Evolution* 4: 341–364.

Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612–622.

Palero, F., Crandall, K.A., Abelló, P., Macpherson, E. and Pascual, M. 2009. Phylogenetic relationships between spiny, slipper and coral lobsters (Crustacea, Decapoda, Achelata). *Molecular Phylogenetics and Evolution* 50: 152–162.

Pascual, J., Senar, J.C. and Domènech, J. 2014. Plumage brightness, vigilance, escape potential, and predation risk in male and female Eurasian Siskins (*Spinus spinus*). *The Auk* 131: 61–72.

Pätzold, R. 2003. *Kompendium der Lerchen Alaudidae.* Dresden: Jan Schimkat Medienpublizistik.

Payne, R.B. 1986. Bird songs and avian systematics. Edited by Johnston, R.F. (ed.). *Current Ornithology* 3: 87–126.

Pedersen, A.B., Jones, K.E., Nunn, C.L. and Altizer, S.A. 2017. Infectious disease and mammalian extinction risk. *Conservation Biology* 21: 1269–1279.

Peres–Neto, P.R., Jackson, D.A. and Somer, K.M. 2005. How many principal components? Stopping Rules for determining the number of Non–trivial axes revisited. *British Journal of Statistical Psychology* 49: 974–997.

Perrins, C. 2003. *Firefly Encyclopedia of Birds.* Firefly Books.

Peters, J.L. 1960. “Family Alaudidae.” In *Checklist of birds of the world*, by Mayr, E. and Greenway, J.C.J. (eds.). pp. 55. Cambridge, MA: Museum of Comparative Zoology.

Piperno, E. and Peirone, S. 1975. Morphological characteristics and mutual relationships of the tracheal cartilaginous rings in *Gallus gallus*. *Anatomia, Histologia, Embryologia* 4: 172–178.

Plazzi, F., Ferrucci, R.R. and Passamonti, M. 2010. Phylogenetic representativeness: a new method for evaluating taxon sampling in evolutionary studies. *BMC Bioinformatics* 11: 209.

Plótnick, R. 1958. Posicion sistematica del genero "*Psilorhamphus*". *Physis* 21: 130–136.

Podos, J. and Moseley, D. 2010. Vocal communication in birds. *Encyclopedia of Neuroscience* 10: 389–396.

Podos, J. and Nowicki, S. 2004. Beaks, adaptation, and vocal evolution in Darwin’s finches. *Bioscience* 54: 501–510.

Podos, J. 2007. Discrimination of geographical song variants by Darwin’s finches. *Animal Behaviour* 73: 833–844.

Podos, J., Lahti, D. and Moseley, D. 2009. Vocal performance and sensorimotor learning in songbirds. *Advances in the Study of Behavior* 40: 159–195.

Price, J.J. and Lanyon, S.M. 2002. Reconstructing the evolution of complex bird song in the oropendolas. *Evolution* 56: 1514–1529.

Prince, B., Riede, T. and Goller F. 2011. Sexual dimorphism and bilateral asymmetry of syrinx and vocal tract in the European Starling (*Sturnus vulgaris*). *Journal of Morphology* 272: 1527–1536.

Prum, R.O. and Lanyon, W.E. 1989. Monophyly and phylogeny of the *Schiffomis* group (Tyrannoidea). *Condor* 91: 444–461.

Prum, R.O. 1992. Syringeal morphology, phylogeny, and evolution of the Neotropical manakins (Aves: Pipridae). *American Museum Novitates* 3043: 1–65.

Puillandre, N., Modica, M.V., Zhang, Y., Sirovich, L., Boisselier, M.C., Cruaud, C., Holford, M. and Samadi, S. 2012. Large–scale species delimitation method for hyperdiverse groups. *Molecular Ecology* 21 (11): 2671–2691.

Pycraft, W.P. 1905. On the systematic position of *Zeledonia coronata*, with observations on the position of the Turdidae. *International Journal of Avian Science* 47: 1–24.

R Core Development. 2018. *R: A language and environment for statistical computing.* Austria: R Foundation for Statistical Computing Vienna.

Raposo, M.A. and Höfling, E. 2003. Overestimation of vocal characters in Suboscine taxonomy (Aves: Passeriformes: Tyranni): Causes and implications. *Lundiana* 4 (1): 35–42.

Reid, J.M.P., Arcese, A.L., Cassidy, E.V., Hiebert, S.M., Smith, J.N., Stoddard, P., Marr, B. and Keller, L.F. 2005. Fitness correlates of song repertoires size in free living song sparrow (*Melospiza melodia*). *The American Nauralist* 165 (3): 299–310.

Rendall, D. and Kaluthota, C.D. 2013. Song organization and variability in Northern House Wrens (*Troglodytes aedon parkmanii*) in western Canada. *The Auk* 130: 617–628.

Reyes, E., Nadot, S., von Balthazar, M., Schönenberger, J. and Sauquet, H. 2018. Testing the impact of morphological rate heterogeneity on ancestral state reconstruction of five floral traits in angiosperms. *Scientific Reports* 8 (9473).

Ricklefs, R.E. 2007. Estimating diversification rates from phylogenetic information. *Trends in Ecology and Evolution* 22 (11): 601–610.

Riede, T., Fisher, J.H. and Goller, F. 2010. Sexual dimorphism of the zebra finch syrinx indicates adaptation for high fundamental frequencies in males. *PloS ONE* 5 (6): e11368.

Roberts, A. 1940. *The birds of South Africa.* London: H.F. & G. Witherby.

Robillard, T. and Dessuter–Grandcolas, L. 2004. High–frequency calling in Eneopterinae crickets (Orthoptera, Grylloidea, Eneopteridae): adaptative radiation

revealed by phylogenetic analysis. *Biological Journal of the Linnean Society* 83: 577–584.

Robin, V.V., Sinha, A. and Ramakrishnan, U. 2010. Ancient geographical gaps and paleo–climate shape the phylogeography of an endemic bird in the sky islands of southern India. *PLoS ONE* 5: e13321.

Ronquist, F. 2004. Bayesian inference of character evolution. *Trends in Ecology and Evolution* 19 (9): 475–481.

Royer-Carenzi, M. and Didier, G. 2015. A comparison of Ancestral state reconstruction methods for quantitative characters. *bioRxiv* 037812.

Ruiz, C., Jordal, B. and Serrano, J. 2009. Molecular phylogeny of the tribe Sphodrini (Coleoptera: Carabidae) based on mitochondrial and nuclear markers. *Molecular Phylogenetics and Evolution* 50: 44–58.

Rüppell, W. 1933. Physiologie und Akustik der Vogelstimme. *Journal für Ornithologie* 81: 433–542.

Ryan, P.G. and Bloomer, P. 1999. The Long–billed complex: a species mosaic in south–western Africa. *The Auk* 116: 194–208.

Ryan, P.G. and Marshall, H.K. 2005. Variation in the wing–clapping display of Clapper Larks. *Ostrich: Journal of African Ornithology* 76 (1/2): 73–77.

Ryan, P.G., Hood, I., Bloomer, P., Komen, J. and Crowe, T.M. 1998. Barlow's Lark: a new species in the Karoo Lark Certhilauda albescens complex of southwest Africa. *International Journal of Avian Science* 140: 605–619.

Saranathan, V., Hamilton, D., Powell, G.V.N., Kroodsma, D.E. and Prum, R.O. 2007. “Genetic evidence supports song learning in the three–wattled bellbird *Procnias tricarunculata* (Cotingidae). *Molecular Ecology* 16: 3689–3702.

Savart, F. 1826. Memoire sur la voix des oiseaux. *Annales de chimie et de physique* 32: 5–24.

Sayers, E.W., Barrett, T., Benson, D.A., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M. and DiCuccio, M. 2009. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research* 37: 5–15.

Schaefer, H., Hechenleitner, P., Santos–Guerra, A., de Sequeira, M.M., Pennington, R.T., Kenicer, G. and Carine, M.A. 2012. Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle–Atlantic island lineages. *BMC Evolutionary Biology* 12: 250.

Schwartz, J.H. 2011. Systematics and evolutionary biology: uneasy bedfellows? *Revista Mexicana de Biodiversidad* 82: 1–18.

Sclater, P.L. 1888. *Catalogue of the birds of the British Museum.* Vol. 14. London: Trustees of the British Museum.

Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical Suboscine birds. *Evolution* 59: 200–215.

Selvatti, A.P., Galvão, A., Pereira, A.G., Gonzaga, L.P. and de Moraes Russo, C.A. 2017. An African origin of the Eurylaimides (Passeriformes) and the successful diversification of the ground-foraging pittas (Pittidae). *Molecular Biology and Evolution* (forthcoming).

Seneviratne, S.S., Jones, I.L. and Carr, S.M. 2012. Patterns of vocal divergence in a group of non–oscine birds (auklets; Alcidae, Charadriiformes). *Evolutionary Ecology Research* 14 (1): 95–112.

Setterwall, G.G. 1901. *Studier of ver Syrinx hos Polymyoda Passeres.* Akad. Lund: Afhandl.

Sibley, C.G. and Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds: A Study in Molecular Evolution.* New Haven and London: Yale University Press.

Sibley, C.G. and Ahlquist, J.E. 1985. The phylogeny and classification of the Australo–Papuan passerine birds. *Emu – Austral Ornithology* 85: 1–14.

Sibley, C.G. and Monroe, B.L., Jr. 1990. *Distribution and taxonomy of birds of the World.* New Haven: Yale University Press.

Simpson, M.G. 2010. *Plant Systematics.* 2nd edition. Massachusetts: Academic Press.

Sinclair, I. and Ryan, P. 2003. *Birds of Africa South of Sahara.* Cape Town: Struik Publishers.

Snow, D.W. 1970. A field study of the bearded bellbird in Trinidad. *International Journal of Avian Science* 112: 299–329.

Specht, R. 2017. *Avisoft SASLab Pro, Sound Analysis and Synthesis Laboratory software for Windows.* Berlin: Avisoft Bioacoustics.

Spector, D.A. 1994. Definition in biology: the case of bird song. *Journal of Theoretical Biology* 168: 373–381.

Stein, R.C. 1968. Modulation in bird sound. *The Auk* 85 (2): 229–243.

Suárez, F., Hervás, I. and Herranz, J. 2009. *The larks of peninsular Spain: General Directorate for Biodiversity.* Madrid: Ministry of Environment and Rural and Marine Affairs.

Suthers, R.A. 1994. Variable asymmetry and resonance in the avian vocal tract: A structural basis for individually distinct vocalizations. *Journal of Comparative Physiology A* 175: 457–466.

Suthers, R.A., Goller, F. and Pytte, C. 1999. The neuromuscular control of birdsong. *Philosophical Transactions of the Royal Society London B* 354: 927–939.

Swofford, D.L. 2002. *Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0 Beta 10.* Sunderland: Snauer Associates.

Thielcke, G. 1970. Lernen von gesang als möglicher schrittmacher der evolution. *Journal of Zoological Systematics and Evolutionary Research* 8 (1): 309–320.

Thompson, K.A. and Newmaster, S.G. 2014. Molecular taxonomic tools provide more accurate estimates of species richness at less cost than traditional morphology–based taxonomic practices in a vegetation survey. *Biodiversity Conservation* 23: 14.

Tieleman, B.I., Williams, J.B. and Bloomer, P. 2003. Adaptation of metabolism and evaporative water loss along an aridity gradient. *Proceedings of the Royal Society of London, Series B* 270: 207–214.

Tietze, D.T., Martens, J., Sun, Y.H. and Päckert, M. 2008. Evolutionary history of treecreeper vocalisations (Aves: Certhia). *Organisms Diversity and Evolution* 8 (4): 305–324.

Tinbergen, N.1951. *The study of instinct.* Clarendon Press/Oxford University Press.

Tinbergen, N. 1959. Behaviour, systematics, and natural selection. *International Journal of Avian Science* 101 (3–4): 273–525.

Titus, K., Mosher, J.A. and Williams, B.K. 1984. Chance–corrected classification for use in discriminant analysis: ecological applications. *American Midland Naturalist* 111: 1–7.

Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D.C. and Collar, N.J. 2010. Quantitative criteria for species delimitation. *International Journal of Avian Science* 152: 724–746.

Touchton, J.M., Seddon, N. and Tobias, J.A. 2014. Captive rearing experiments confirm song development without learning in a tracheophone Suboscine bird. *PLoS ONE* 9: e95746.

Tsui, C.K.M., Marshall, W., Yokoyama, R., Honda, D., Lippmeier, J.C., Craven, K.D., Peterson, P.D. and Berbee, M.L. 2009. Labyrinthulomycetes phylogeny and its implications for the evolutionary loss of chloroplasts and gain of ectoplasmic gliding. *Molecular Phylogenetic Evolution* 50: 129–140.

Tsukahara, N., Yang, Q. and Sugita, S. 2008. Structure of the syringeal muscles in Jungle Crow (*Corvus macrorhynchus*). *Anatomical Science International* 83: 152–158.

Uchida, A.M., Meyers, R.A., Cooper, B.G. and Goller, F. 2010. Fibre architecture and song activation rates of syringeal muscles are not lateralized in the European starling. *Journal of Experimental Biology* 213: 1069–1078.

Van Niekerk, J.H. 2013. Vocal structure, behavior and partitioning of all 23 *Pternistis* spp. into homologous sound (and monophyletic) groups. *Chinese birds* 4 (3): 210–231.

Veney, S.L. and Wade, J. 2005. Post–hatching syrinx development in the zebra finch: An analysis of androgen receptor, aromatase, estrogen receptor a and estrogen receptor b mRNAs. *Journal of Comparative Physiology A* 191: 97–104.

Veney, S.L. and Wade, J. 2004. Steroid receptors in the adult zebra finch syrinx: A sex difference in androgen receptor mRNA, minimal expression of estrogen receptor and aromatase. *General and Comparative Endocrinology* 136: 192–199.

Verheyen, R. 1958. Contribution a l'anatomie de base et la systematique des Alaudidae (Passeriformes). *Alauda* 26: 1–25.

Vicq d'Azyr, F. 1779. De la structure des organes qui servent a. la formation de la voix, considérés dans l'homme et dans les différents classes d'animaux, et comparés entre eux. *Academie des Sciences Paris, Mémoires* 81: 5–8.

Voelker, G. 1999. Molecular evolutionary relationships in the avian genus Anthus (pipits: Motacillidae). *Molecular Phylogenetics Evolution* 11: 84–94.

Wade, J. and Buhlman, L. 2000. Lateralization and effects of adult androgen in a sexually dimorphic neuromuscular system controlling song in Zebra Finches. *Journal of Comparative Neurology* 426: 154–164.

Wade, J., Buhlman, L. and Swender, D. 2002. Post–hatching hormonal modulation of a sexually dimorphic neuromuscular system controlling song in Zebra Finches. *Brain Research* 929: 191–201.

Weary, D.M., Norris, K.J. and Falls, J.B. 1990. Song feature use to identify individuals. *The Auk* 107: 623–625.

Wetmore, A. and Swales, B.H. 1931. The birds of Haiti and the Dominican Republic. *Bulletin of the United States National Museum* 155: 483.

Wetmore, A. 1943. The birds of southern Vera Cruz, Mexico. *Proceedings of the United States National Museum* 93: 215–340.

White, C.M.N. 1961. *A revised checklist of African broadbills, pitas, larks, swallows, wagtails and pipits.* Lusaka: Government Printer.

Whitehead, A. 20092009. Comparative mitochondrial genomics within and among species of killfish. *BMC Evolutionary Biology* 9: 11.

Whitney, B.M., Pacheco, J.F., Buzzetti, D.R.C. and Parrini, R. 2000. Systematic revision and biogeography of the *Herpsilochmus pileatus* complex, with description of a new species from north–eastern Brazil. *The Auk* 117: 869–891.

Whitney, B.M., Pacheco, J.F., Islerand, P.R. and Isler, M.L. 1995. *Hylopezus nattereri* (Pinto, 1937) is a valid species (Passeriformes: Formicariidae). *Ararajuba* 3: 37–42.

Wiens, J.J. 2004. The role of morphological data in phylogeny reconstruction. *Systematic Biology* 53: 653–661.

Wiley, E.O. 1988. Vicariance biogeography. *Annual Review of Ecology, Evolution, and Systematics* 19: 513–554.

Winterbottom, J.M. 1962. Systematic notes on birds of the Cape Province 18. Comments on the phylogeny of the Alaudidae. *Ostrich: Journal of African Ornithology* 33: 41–42.

Wolters, H.E. 1979. *Die Vogelarten der Erde.* Vol. 4th. Germany: Paul Parey, Hamburg and Berlin.

Wunderlich, L. 1886. Beitrage zur vergleichen Anatomie und Entwicklungsgeschichte desunteren Kehlkopfes der Vogel. *Kaiserlich Leopoldinischen–Carolinischen Deutschen Akad* 18.

Zhao, M., Alström, P., Olsson, U., Qu, Y. and Lei, F. 2016. Phylogenetic position of the Wallcreeper *Tichodroma muraria*. *Journal of Ornithology* 913–918.

Zimmer, K.J., Robbins, M.B. and Kopuchian, C. 2008. Taxonomy, vocalizations, syringeal morphology and natural history of *Automolus raraime* (Furnarridae). *Bulletin of the British Ornithologists' Club* 128: 14–33.

Zimmer, K.J., Whittaker, A. and Oren, D.C. 2001. A cryptic new species of flycatcher (Tyrannidae: *Suiriri*) from the Cerrado region of central South America. *The Auk* 118: 56–7856–78.

Zou, Z. and Zhang, J. 2016. Morphological and molecular convergences in mammalian phylogenetics. *Nature Communications* (Nature Publishing Group) 7: 1–9.