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**Lineage to landscape: systematics and evolution of Asian
camel crickets (Rhaphidophoridae)**

A thesis presented in fulfilment of the requirements for
the degree of

Doctor of Philosophy
in
Conservation Biology

at Massey University, Manawatū,
New Zealand.

Cheten Dorji

2025

To my loving wife and two daughters.



Thesis Abstract

Rhaphidophoridae (camel crickets) comprises nine subfamilies, with three subfamilies and over half of the global diversity found in Asia. Our current understanding of phylogenetic relationships and the evolutionary history of Asian rhaphidophorid lineages remain significantly underexplored. My thesis addresses this gap by investigating the systematic, phylogenetic, and population structures of Asian lineages, leveraging the least explored Himalaya region with additional representatives from the Pacific (Japan, Solomon Islands, and New Zealand) for comparison. Morphological traits and mitochondrial DNA Cytochrome Oxidase I (COI) were used for systematics and phylogeographic analysis of cave associated camel crickets, while whole mitochondrial genome sequences were used to infer phylogeny and timing of the radiation of Asian lineages. I discovered and described nine new species from Eastern Bhutan. My findings indicate that (1) true species diversity of Rhaphidophoridae in the Himalayan region is undoubtedly much higher, considering my discoveries were from a small area of the full territory; (2) the two subfamilies, Rhaphidophorinae and Aemodogryllinae, that overlap in Asia potentially diverged through a series of dispersal events from Gondwana to South East Asia, rather than from walking over the Bering land bridge as previously hypothesized; and (3) the high level of genetic differentiation observed among populations of cave associated camel crickets suggest that they might be primarily cave dwellers potentially limited by climatic, biological and geophysical factors during the Pleistocene. Overall, this study provides an insight into lineage to landscape history of least explored groups of species from underrepresented regions. In conclusion, Asian lineage representation is largely neglected in past studies partly attributable to lack of specimens; robust inclusion of samples from this region in molecular studies will help better understand the long-debated lineage diversification and origin hypotheses of this widely distributed wingless orthoptera.

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Translated into Dzongkha (national language of Bhutan) as accurate as possible with assistance from Mr. Sonam Tenzin (SAARC Cultural Centre, Colombo, Sri Lanka/ Cultural specialist, Department of Culture and Dzongkha Development, Ministry of Home Affairs, Bhutan) and Dr. Tandin Gyeltshen (Lecturer, College of Natural Resources, Royal University of Bhutan).

Declaration by Author

The research carried out for my doctoral thesis has been used in whole or in part, solely for the purpose of this qualification. To the best of my knowledge this thesis contains no material previously published by any other person except where due acknowledgement has been made. The Statement of Contribution to Doctoral Thesis (DRC16 form) has been completed for all published and under-review chapters within this thesis and is included in the electronic copy at the start of each relevant chapter. I have clearly stated the contribution of others in jointly authored works, details of which can be found in DRC16 form.

This thesis is based on publications, and the formatting style of each chapter follows the guidelines for the journals in which it has been published or submitted for publication. However, citations and references have been formatted consistently across all chapters in APA style. Some inconsistencies in writing style and nomenclature may be present. For example, as published papers are co-authored, I have used “we” and “our” in these chapters to be inclusive of co-authors. Different common names for Rhaphidophoridae are used synonymously based on the requirements of the journals, such as cave wētā, cave crickets, camel crickets, camelback crickets, Rhaphs, and Rhaphidophorids.

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My deepest gratitude goes to Steve and Mary, whose kindness and humility have left a lasting cast on my heart. As supervisors, they redefined my understanding of mentorship by being compassionate and approachable. Coming from a different background, I have always pictured supervisors as distant and strictly academic, but Steve and Mary broke that barrier, showing me that empathy and support is all it takes to create a conducive and inspiring learning environment. I have learnt invaluable lessons from them, and I hope to carry this forward and inspire my own students back home.

A major slice of this accomplishment goes to my wife and our two lovely girls, who made countless sacrifices during the years we spent apart. Though distance separated us, it brought us together and ultimately strengthened our bond, and I am forever grateful for their love and patience.

I never had an opportunity to express gratitude to my parents for their unwavering support and love they have shown me throughout my life. From the very beginning, they believed in my education and encouraged me at every step, no matter the challenges. Words cannot fully capture the depth of my appreciation for the impact they have had on my life journey. I know I can never repay them for everything they have done, but I am eternally grateful to have such wonderful parents. Thank you, Mom and Dad, for your endless love and guidance.

This journey was not a solitary one; my time in New Zealand was brightened by the friendship and encouragement of many. I am grateful to my friends in the ecology group—Boyoung, Elina, Gillian, Jesper, Kwang-Sik, Leo, Mari, Nysha, Nim, Olivia, Shogo, Simon, Xiaonchuan and as well as to the laboratory technicians (Cleland, Shaun, and Tracy) for their invaluable support and constructive feedback. I also thank my distant friends and families who kept me connected through messages and calls.

I extend sincere appreciation to the Royal University of Bhutan and the Royal Government of Bhutan for their financial and institutional support, without which this professional journey would not have been possible. My gratitude also goes to the College of Natural Resources for granting me study leave and to all my colleagues for supporting my academic growth. Finally, a special thanks to Dr. Phub Dorji, the late President of the College of Natural Resources and Professor Dr. D.B Gurung, whose mentorship and encouragement were instrumental in my career.

“Namay Samay Kadrinchy” which translate to
“Thank you beyond the sky and the earth”
for being part of this incredible journey.



Publications During Candidature

Peer-reviewed journal article, included in this thesis:

Chapter One

Dorji, C., & Trewick, S. A. (2025). *Caves without cave crickets in Bhutan.* pp 147–139 in: Husemann M, Hawlitschek, O (eds). Grasshoppers, locusts, and crickets of the World. Princeton University Press: Princeton, Princeton and Oxford.

Chapter Two

Dorji, C., Morgan-Richards, M. & Trewick, S.A. (2025) Little-known wingless crickets of Bhutan (Rhopidophoridae): discovery and description of nine new species. *Zootaxa*, 5653 (1), 1–32. <https://doi.org/10.11646/zootaxa.5653.1.1>

Chapter Three

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PREFACE

The overarching aim of this research project was to explore the systematics, phylogeny, and phylogeography of wingless Orthoptera (Rhaphidophoridae) with a focus on the fauna of the Kingdom of Bhutan. Due to limited study of this family in Bhutan, it was necessary to start with sampling and describing the diversity I found. Half a century since the only previous study of Orthoptera in Bhutan by the Natural History Museum of Basel in 1972, I undertook for the first time, a systematic survey of caves and nearby forests for Rhaphidophoridae (camel crickets). Caves and caverns are culturally significant pilgrim sites for Bhutanese Buddhists and also unique habitats supporting diverse organisms adapted to low-light environments. Chapter 1 of this thesis presents first-hand observation of camel crickets and considered their association with caves and the culture of Bhutan. This was published in 2025 as a chapter in the international volume “Grasshoppers, Locusts, and Crickets of the World” (Dorji & Trewick, 2025).

Despite Rhaphidophoridae have received extensive research attention around the world in the past five decades, significant gaps remain in taxonomy, ecology and molecular studies from south and southeast Asia. Asia is the stronghold of two major subfamilies (Aemodogryllinae and Rhaphidophorinae), with a third (Anoplophilinae) being recorded only in Korea and Japan. Current taxonomic understanding of these groups in Asia relies almost entirely on morphological characters with no comprehensive molecular framework available for systematic classification (e.g., Bian et al., 2017; Dawwrueng et al., 2020; Qin et al., 2016). Chapter 2 uses an integrated approach combining both morphological and molecular data to investigate the systematics and diversity of camel crickets in Bhutan, based on new sampling and comparison with published data. This chapter aims to (1) identify and describe species level diversity among camel crickets in Bhutan, (2) validate the current taxonomic placement of genera within the Rhaphidophorinae and Aemodogryllinae subfamilies, and (3) validate morphological trait-based classification of subgenera in *Diestramima* (or groups in *Rhaphidophora*). Integrative molecular and morphological approaches for taxonomy and validation of classifications not only help resolve long-standing taxonomic ambiguities, but also enable accurate identification and discovery, particularly where traditional morphological methods are challenging.

Fossil dating of sister Ensifera and molecular phylogenetic analysis implies an origin of the Rhaphidophoridae variously estimated as between the upper Carboniferous and the Jurassic, with continental drift vicariance and long-distance dispersal implicated in the present-day

PREFACE

biogeography of the family (e.g., Allegrucci & Sbordoni, 2019; Ander, 1939; Hubbell & Norton, 1978; Kim et al., 2024). The phylogenomic analysis of Asian lineage remains largely unexplored, though recent family-level phylogenetic studies based on secondary calibration predict Cretaceous origin linked to the Bering Land Bridge. Chapter 3 tests this hypothesis by reconstructing a fossil-calibrated molecular phylogeny of Asian Rhaphidophoridae using complete mitochondrial genome sequences. This chapter aims to (1) estimate the origin of Asian lineages, (2) determine the most plausible scenarios that may explain their current distribution patterns, and (3) discuss the taxonomic position of Asian representative genera based on phylogenomic inference. The Rhaphidophorinae and Aemodogryllinae overlap in Bhutan and extend from Asia into the geologically complex archipelago of the south Pacific, and by extending sampling and using fossil-calibrated phylogenetic analysis, better insight into the roles of long-distance dispersal and palaeogeography in shaping regional diversity is inferred.

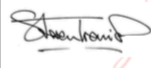
Population structure is fundamental to understanding the evolution of cave-dwelling organisms, because it represents the outcome of their history and adaptation as well as forms the basis for speciation in the cave environment (Sbordoni et al., 2012). Rhaphidophoridae adapted to cave systems serve as models for studying pattern of genetic diversity and often represent contrasting levels of genetic diversity that suggest different timing of colonisation, population size and/or movement among caves. Chapter 4 assesses the extent of genetic connectivity across small geographic scales to determine whether cave samples act as discrete populations. I generate and analyse new mitochondrial COI DNA from cave-associated species in Maniapoto karst, New Zealand, and limestone caves from Okinawa, Japan. This chapter aims to (1) analyse the degree of genetic variation among cave-associated camel crickets, (2) examine the influence of geological history, paleoclimatic and dispersal ability on distribution of genetic variation, and (3) compare the phylogeographical structure among cave and forest-dwelling species from around the world using available genetic data. Differences in dispersal ability and ecology, and landscape history provide a framework for understanding their effects on the phylogeographical structure and isolation of cave-dwelling populations.

In chapter 5 and final chapter, I summarise the major findings of this thesis and present preliminary results from Ecological Niche Modelling (ENM), a powerful tool for predicting climate-driven range shifts using species occurrences and climatic variables (Briscoe et al., 2019; Dormann et al., 2012; Wiens et al., 2009). Focusing on two New Zealand camel cricket species, *Pharmacus cochleatus* and *P. montanus*, that live in the Southern Alps of South Island, I will investigate how current distribution, shaped by Pleistocene glacial cycling, can be

projected under various climate scenarios. By modelling climate envelopes, I will assess potential range shifts and infer past and present climatic impacts on their distributions and discuss the use of these approaches in future study of Rhaphidophoridae. Replicating these types of studies with the biodiversity of Bhutan will be possible when location data for newly described species has been expanded. Given the steep elevation gradient in Bhutan and the rapidly changing global climate, tracking species range shifts will be important future work that follows this thesis.

STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

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Name and title of main supervisor:	Steven A. Trewick		
In which chapter is the manuscript/published work?	One		
Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:¹ Cheten Dorji: Conceptualization, writing original draft, and figure visualization, and formatting. Steven A. Trewick: Conceptualization, supervision, reviewing, editing.			
Please select one of the following three options:			
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Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:¹ Cheten Dorji: Conceptualization, collection of biological material and generation of DNA sequences, analysis, writing original draft, and photography. Steven A. Trewick and Mary Morgan-Richards: Conceptualization, supervision, resource facilitation, reviewing, editing,	
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CHAPTER TWO

Little-known wingless crickets of Bhutan (Rhaphidophoridae): discovery and description of nine new species.

ABSTRACT

We present the results of a survey of raphidophorids in Bhutan more than half a century since the first systematic exploration of Orthoptera by the Natural History Museum Basel Expedition to Bhutan in 1972. Only one of two species previously described from Bhutan was resampled. A total of nine new species are described belonging to the family Rhaphidophoridae: five species in genus *Diestramima* Storozhenko (subfamily Aemodogryllinae), and four species in genus *Rhaphidophora* Serville (subfamily Rhaphidophorinae). We provide details of morphological structures for all newly described species and their known distribution and habitat. The phylogenetic relationships of the raphidophorids from Bhutan are inferred from mitochondrial DNA sequences (COI). New species of *Diestramima* are not assigned to subgenera because the shape of their paraproct is intermediate when compared to the shapes described for each subgenera. Keys to the Bhutan species based on male epiproct (*Rhaphidophora*) and posteromedian projection of 7th abdominal tergite (*Diestramima*) are provided. New species are *Diestramima matermagna* **sp. nov.**, *D. minjiwoonga* **sp. nov.**, *D. multidenticuli* **sp. nov.**, *D. samkhara* **sp. nov.**, and *D. phubdorjia* **sp. nov.**; *Rhaphidophora bhutanensis* **sp. nov.**, *R. bicuspis* **sp. nov.**, *R. bilobata* **sp. nov.**, and *R. piscicauda* **sp. nov.**

KEYWORDS

Diestramima, *Rhaphidophora*, phylogeny, cave cricket, camel crickets, raphidophorids.

INTRODUCTION

Rhaphidophorids (Camel crickets) are wingless Ensifera with undeveloped stridulatory structures. They are nocturnal, usually restricted to dark and moist environments and often associated with caves. Most species are capable of climbing tree trunks and rocks, and some survive winter freezing (Hegg et al., 2022; Richards, 1965). The family Rhaphidophoridae has a global distribution with subfamilies restricted to particular geographic regions (Kim et al., 2024). Of nine subfamilies, three are found in Asia, primarily inhabiting tropical regions of southeast Asia (Fig. 1A). The subfamily Aemodogryllinae is found from eastern Russia to east and southeast Asia, while the related subfamily Rhaphidophorinae is more southern with a distribution stretching from east Asia and central China to the Samoan Archipelago (Gorochov & Storozhenko, 2015) (Fig. 1A). Both Aemodogryllinae and Rhaphidophorinae are recorded in temperate and tropical forests of Bhutan (Ingrisch, 2002; Würmli, 1973). Subfamily Anoplophilinae is restricted to Korea and Japan (Fig. 1A).

Molecular phylogenetic studies support the division of the family Rhaphidophoridae into nine subfamilies (Kim et al., 2024). However, current taxonomy at the level of tribe and genus is largely based on morphology, often using traits only possessed by adult males (e.g. Dawwrueng et al., 2020, 2020; Gorochov, 2010; Qin et al., 2018). The tribe Diestramimini of subfamily Aemodogryllinae consists of seven genera (Gorochov & Storozhenko, 2015), of which *Tamdaotettix* and *Mimadiestra* are characterized by a spine-like posteromedian projection on the 6th abdominal tergite and a pair of deeply divided spines on the 7th abdominal tergite, respectively. *Gigantettix* and *Adiestramima* are differentiated from *Diestramima* and *Megadiestramima* by the presence of ventral spines on the fore femora. Whereas *Diestramima* is easily distinguishable from other genera of this tribe by the presence of a long posteromedian process of the 7th abdominal tergite that completely covers the paraprocts and by the eight lobed membranous genitalia. The only monotypic genus *Arboramima* is differentiated from others by “dark green and moss like” coloration, found inhabiting trees covered with moist moss (Zong et al., 2021). Further division of *Diestramima* into three subgenera (*Baculitettix* n=11, *Diestramima* n=6, and *Excisotettix* n=5) has been proposed on the basis of paraproct shape (Gorochov & Storozhenko, 2019).

Genera in subfamily Rhaphidophorinae are morphologically homogeneous. Six of the eight genera are characterised by the position of posteromedian projections on abdominal tergites 6 to 10. The other two, *Rhaphidophora* and *Neorhaphidophora*, lack posteromedian projections

on abdominal tergites. *Rhaphidophora* is the most speciose genus in the subfamily with >150 species across Asia and Oceania (Cigliano et al., 2023). *Rhaphidophora* males have a simple or weakly specialized epiproct bearing a process or a curved, narrowed, or bifurcate apical area (Bian & Shi, 2016; Gorochoy, 2011) and few distinctive genitalic traits. Five species are recognised in *Neorhaphidophora* based on the specialised shape of the male epiproct (Bian et al., 2017; Dawwrueng et al., 2020a; Gorochoy, 2011).

The kingdom of Bhutan on the southern slopes of the Himalaya is bordered by Tibet and India (Fig. 1A) but is geopolitically relatively isolated. More than 50% of Bhutan's land area has national protected status and about 70% is covered in native forest (Royal Government of Bhutan, 2022). The government of Bhutan imposes strict regulations to prevent the collection of flora and fauna which limits access to biological material by international researchers. These regulations have left Bhutan as a biodiversity hotspot in the eastern Himalaya waiting to be explored by the locals. The only foreign expedition to study Orthoptera in Bhutan was carried out by the Natural History Museum of Basel in 1972. The results published in 1973 included descriptions of a some species of Gryllacridoidea and a single raphidophorid *Diestramima tsongkhapa* (Würmli, 1973). Thirty years later, specimens from the expedition were examined and a checklist of 69 Orthoptera from Bhutan including description of a second raphidophorid *Rhaphidophora angulata* was reported (Ingrisch, 2002). As much of Bhutan is remote and difficult to access there remains much to learn about the biodiversity of this country.

This is the first study of Rhaphidophoridae in Bhutan since the Basel expedition, and Orthoptera were sampled from 15 sites across the country (Fig. 4). We describe nine new raphidophorid species belonging to two well-recognised genera from Asia under two subfamilies. For systematic inference we used morphological traits and molecular phylogenetics. For the first time, more than 45 samples representing Bhutanese raphidophorids are genetically sequenced and their phylogenetic relationships determined.

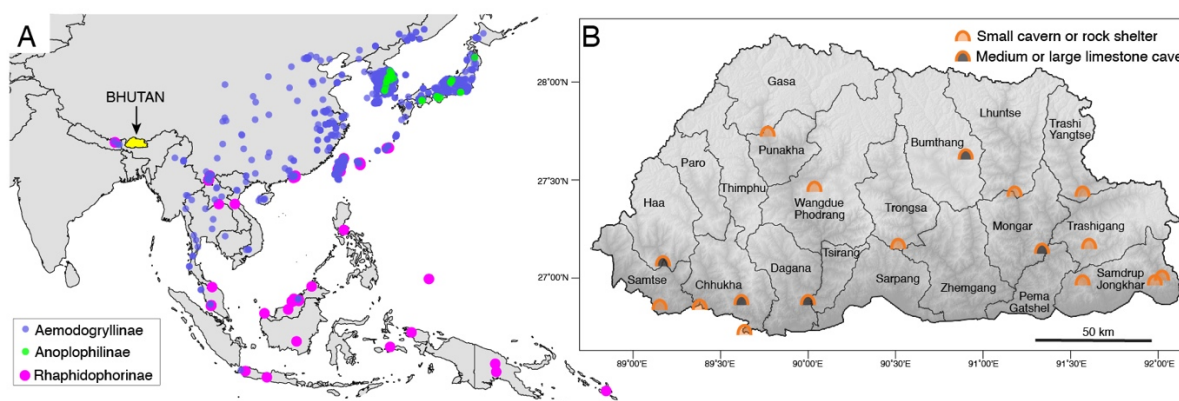


Figure 1. (A) Records of three Rhaphidophoridae subfamilies present in Asia and Oceania, with the Kingdom of Bhutan highlighted in yellow. Data from iNaturalist (<https://www.inaturalist.org/>) and BOLD system (<https://boldsystems.org>) processed in QGIS and Inkscape. (B) Cave systems searched for Rhaphidophoridae among districts (=dzongkhag) of Bhutan.

MATERIALS AND METHODS

Sampling

We explored some of the well-known caves and nearby forest in the remote part of eastern Bhutan (Fig. 1B). Although caves are rare, they have remained unexplored for centuries. Internal cave systems and entrances were investigated during the day for the presence of raphidophorids, and searches were made in nearby forest habitat under leaf litter, fallen logs, crack and crevices, and boulders and rocks. Detailed habitat descriptions are provided under each taxonomic account.

Specimens were collected during April 2022 prior to the onset of monsoon, under the authorization of research permit number 7008186946225A7A8C6D0B granted by the Ugyen Wangchuck Institute for Forestry Research and Training (UWIFoRT), Bhutan. A total of 75 specimens were collected by hand-picking from cave entrances and forest habitats predominantly located in the central and eastern region. To ensure their preservation, all samples were stored in ethanol. Transfer of samples to Massey University, New Zealand was conducted in accordance with the Material Transfer Agreement reference number NBC/BRD/7/2022-2023/197 issued by the National Biodiversity Centre, Serbithang, Thimphu, Bhutan. Currently, all the specimens examined are housed in Phoenix collection, Massey University, Palmerston North (MPN). All type specimens will be housed at the invertebrate repository museum at National Biodiversity Centre, Bhutan (NBCB).



Figure 2. Typical habitats of rhabdophorids in eastern Bhutan. (A) Samdrup Jongkhar: Wangphu, 1028 meter above sea level (m asl), typical subtropical broadleaf forest: *Diestramima matermagna* **sp. nov.** (B) Trashigang: Kharungla, 2038 m asl, grazed open shelter of cool broadleaf forest: *D. matermagna* **sp. nov.** (C) Samdrup Jongkhar: Minjiwoong, 833 m asl, rock cave entrance: *D. minjiwoonga* **sp. nov.** (D) Pema Gatshel: Marung, 571 m asl, under canopy vegetation with abundant of leaf litter: *D. matermagna* **sp. nov.**, *Rhabdophora bhutanensis* **sp. nov.** (E) Trashi Yangtse: Bawoong, 1581 m asl, boulders with surrounding vegetation: *D. multidenticuli* **sp. nov.**

Genetic Data

For haplotype analysis, DNA was extracted from leg muscle tissue using Quantabio (Beverly, MA, USA) Extracta DNA Prep using 30 μ L of the extraction reagent in 1.5 mL microfuge tube containing a small piece of leg and incubated at 95 °C for 15 minutes in a thermal shaker. After incubation and cooling for 5 minutes, 30 μ L of stabilizing solution was added. Segments of the mitochondrial gene cytochrome oxidase I (COI) were amplified using polymerase chain reaction (PCR) with primers C1-J-2195 (Simon et al., 1994) and mtd12_wetaR (Bulgarella et al., 2014) and/or LCO1490 and HCO2198 (Folmer et al., 1994). Each 10 μ L PCR reaction consisted of 1.0 μ L of 10x PCR buffer, 0.8 μ L of 25mM $MgCl_2$, 0.2 μ L of each 2mM dNTP, 0.4 μ L of each 10 μ M primer, 0.08 μ L of DreamTaq DNA polymerase (Thermo Scientific, Waltham, MA, USA), and 1.0 μ L of DNA template. Thermocycling used initial denaturation of 95°C for 90 s with 38 cycles of: 94°C for 15 s, 52°C for 15 s and 72°C for 90 s. Reactions were visualised by electrophoresis on 1% TAE agarose gels and PCR products of expected size were subject to Sanger sequencing. Sequences were checked for insertion/deletions, reading frame

anomalies and ambiguities then aligned in Geneious v.9 (Kearse et al., 2012). Phylogenetic inference used Neighbor-Joining (Saitou & Nei, 1987) in Geneious, and Maximum Likelihood (ML) implemented in IQTree2 (Minh et al., 2020) with the ultrafast bootstrap option (Hoang et al., 2018).

Morphology, Measurement, and Identification

Specimens were examined, measured and photographed under stereo-microscope Olympus XZS7 with an Olympus camera and inbuilt software Olympus Cellsens Dimension v.4.4.4. Final images were processed using Inkscape v.1.3.2. We followed Zhu & Shi (2018) for measurement of external structures with some additions (Table 1, Fig. 3Left). Identification and interpretation of *Diestramima* and *Rhaphidophora* drew upon relevant literature (Dawwrueng et al., 2020a; Gorochoy, 1998, 2001, 2010; Gorochoy & Storozhenko, 1992, 2015, 2019; Liu et al., 2021; Qin et al., 2016; Storozhenko & Dawwrueng, 2014; P. Wang et al., 2019; Zhu & Shi, 2018). Detailed counts of leg spines are presented for each species (Tables 3 & 5; supplementary table S1 & S2). For *Rhaphidophora* species, we explored ventral part of epiproct (here by referred as basal plate) which usually remain inserted in genital cavity. The detail structure illustration and description are also presented.

Table 1. Measurement details and abbreviations used for description of species belonging to genera *Rhaphidophora* and *Diestramima*.

Morphometric	Abbreviation	Description
Body Length	BL	Body Length from fastigium to posterior margin of 10 th abdominal tergite.
	PL	Pronotum Length from anterior to the posterior margin.
Leg Length	FFL/MFL/HFL	Fore/Middle/Hind Femur Length from base to the apex, measured in lateral view.
	FTL/MTL/HTL	Fore/Middle/Hind Tibiae Length from base to the apex.
	FBL/MBL/HBL	Fore/Middle/Hind Basitarsus Length from base to the apex of apical spine.
Eye Width/Length	Eye W/L	Eye Width and its vertical Length from the centre.
Maxillary Palpi Length	MPL	Maxillary Palp Length from base to the apex of each segment.
Subgenital plate Width/Length	Sgp W/L	Subgenital plate lateral Width and Length from base to the apex.
Styli Length	SL	Styli Length from base to the apex (straight line distance on lateral view).
Cerci Length	CL	Cerci Length from base to the apex, measured in later view.
Ovipositor Length	OvL	Ovipositor Length from base to apex (straight line distance on lateral view).

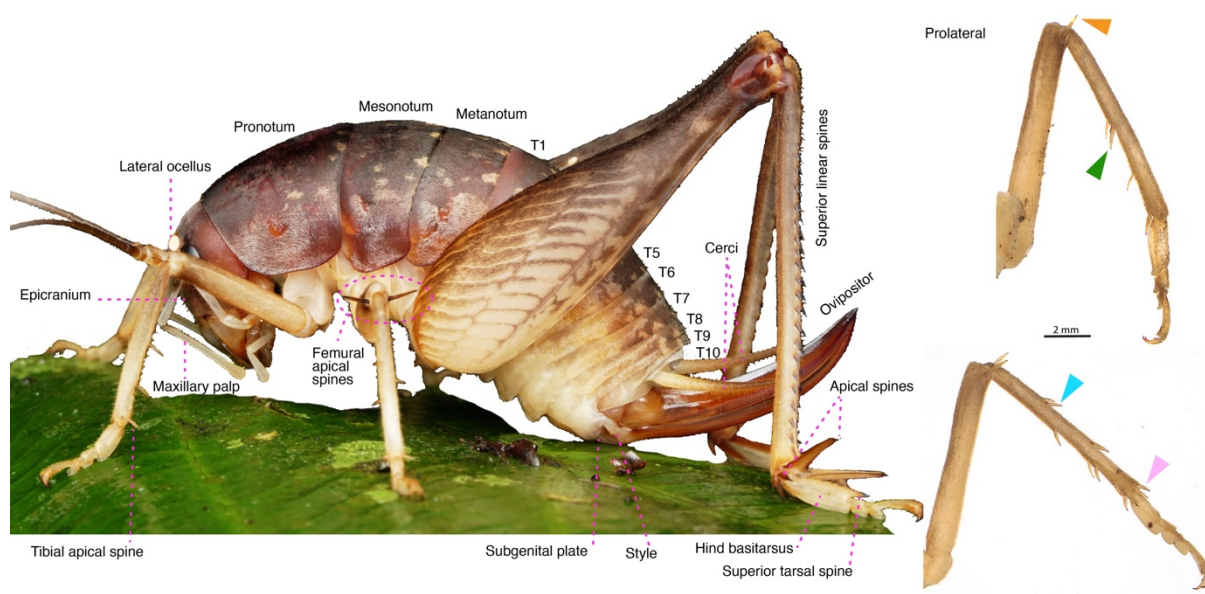


Figure 3. (Left) External structures referred to descriptions of new *Rhaphidophora* and *Diestramima* species. Original image of *Rhaphidophora taiwana* (adult female) by Do-yoon Kim, <https://www.inaturalist.org/people/galois>. (Right) Spine arrangement on fore (top) and mid (bottom) tibiae in prolateral view: prolateral apical spine (orange arrow), inferior linear spines (green arrow), superior linear spines (blue arrow), apical spines (pink arrow).

RESULTS

Distribution and Habitat

Searches of caves and rock shelters at 50 sites across 17 districts of Bhutan (Fig. 1B), in habitats ranging from subtropical to cool broadleaf forest (150–3300 m asl) revealed raphidophorids at 15 sites (Fig. 4). The habitats featured warm, humid tropical and cool, wet temperate climate with abundant canopy vegetation. The highest recorded elevation for raphidophorids was approximately 2800 m asl, where *R. bicuspis* **sp. nov.** was found on the southern aspect of Lamperi (27.498°N, 89.767°E), Thimphu. In contrast, the lowest recorded elevation was 570 m asl, where *D. maternmagna* **sp. nov.** was collected near the Marung bridge in Pema Gatshel (27.040°N, 91.380°E).

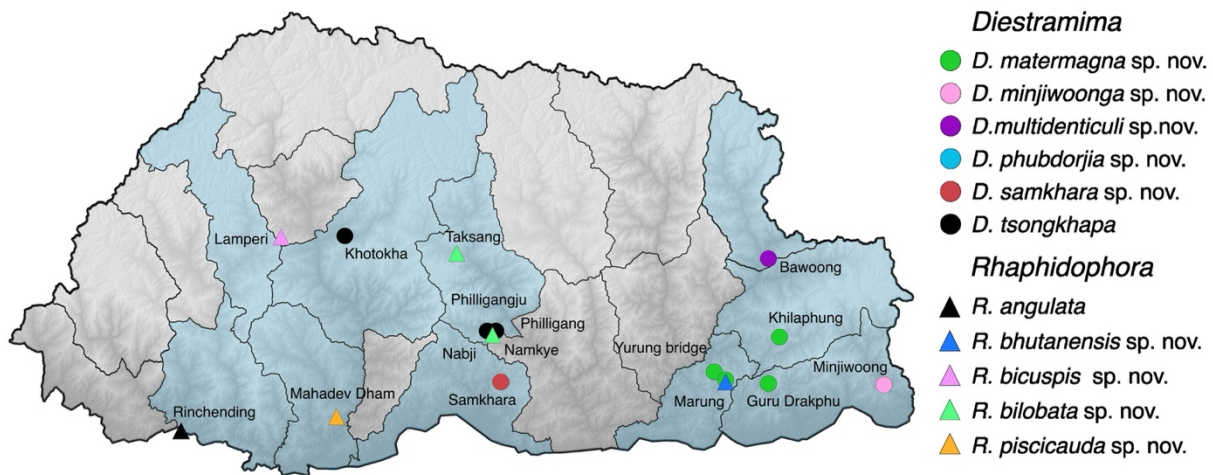


Figure 4. Type localities of nine newly described Rhaphidophoridae from ten districts in Bhutan. *Rhaphidophora angulata* was collected in 1970 but not encountered in the present study.

Systematics

Using mitochondrial DNA (mtDNA) sequences, camel cricket samples from Bhutan clustered to form the two clades *Diestramima* (Aemodogryllinae) and *Rhaphidophora* (Rhaphidophorinae). Within the *Diestramima* clade, six lineages were identified based on genetic clusters and sequence divergence, including a representative of the species *D. tsongkhapa*. These six lineages correspond to distinct morphological differences in the shape of 7th abdominal tergite of males. Based on the concordance of mtDNA variation and morphology we describe five new *Diestramima* species (Fig. 5). Within the Rhaphidophorinae clade, five lineages were identified based on genetic clusters and sequence divergence, including a representative of the species *R. quadrispina* Liu & Bian. These five lineages were represented by individuals with distinct morphology, so we describe here four new species belonging to the well-known Asian genus *Rhaphidophora* (Fig. 13). All genetic data used for phylogenetic analysis can be found in GenBank with accession number PV593656–PV593700.

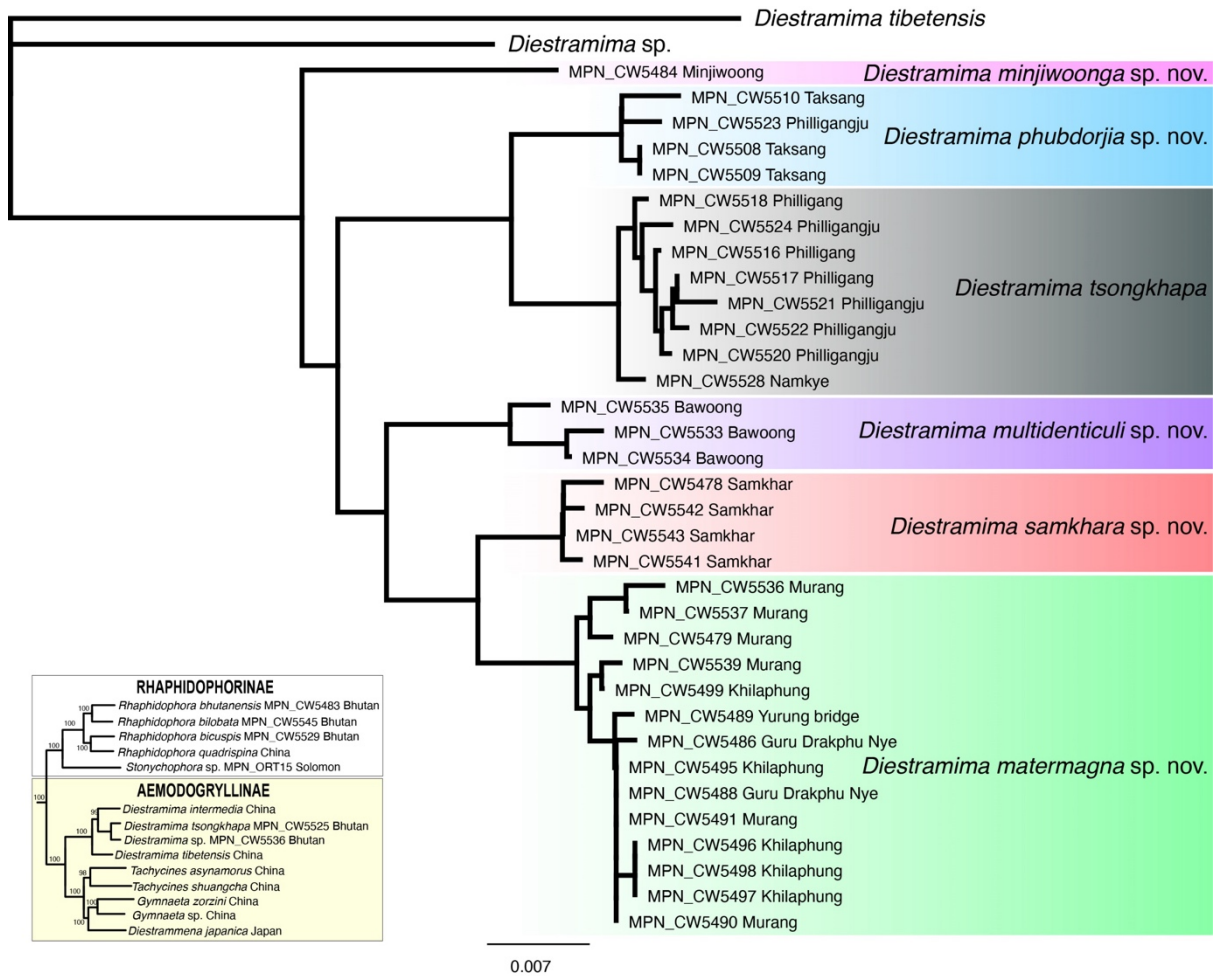


Figure 5. Neighbor-Joining (HKW) phylogeny of *Diestramima* raphidophorids from Bhutan using 780bp alignment of mtDNA COI sequences. Inset: Phylogenetic relationships of genera within subfamily Raphidophorinae and Aemodogryllinae resulting from ML analysis of mtDNA sequences (COI) of 1455 bp.

Subfamily AEMODOGRYLLINAE

Tribe **DIESTRAMIMINI** Gorochoy, 1998

Genus **DIESTRAMIMA** Storozhenko, 1990

Type species: *Diestrammena palpata* Rehn, 1906, by original designation

The genus *Diestramima* was defined by Storozhenko (1990) and has 42 recognised species divided among a number of subgenera (Cigliano et al. 2025). The general characteristics that unite the species within this genus, such as the projection from 7th abdominal tergite of males are described here.

HEAD. Oval in shape. Face smooth and shiny, and usually with short hair on the dorsal surface of head, maxillary palps and antennae. Fastigium vertici always projecting forward, dividing

into apical half of conical tubercles. Bases of tubercles drawn together with obtuse apices. Eyes oval, almost the size of antennal scapi. Median ocellus oval to oblong, and lateral ocelli oval or almost circular situated on lateral surface of basal fastigium vertici.

THORAX. Body medium in size with abundant fine black and brown hairs (Table 2). Anterior margin of the pronotum straight, posterior margin protruding and obtusely rounded, posterolateral margins almost rounded. The posterior margin of mesonotum obviously protruding backward, lateral margins angular; posterior margin of metanotum rather straight with almost circular lateral margins. Posteromedian projection of 7th abdominal tergite variable from short to long covering distal half or entire part of paraproct (Fig. 7 & 8). The shape of the 7th abdominal tergite projection is described under each species account. The paraproct variable with short to long almost finger-like structure (Fig. 7 & 8). The cerci usually slender, conical, and have acute apices.

LEGS. Legs long compared to *Rhaphidophora* (Table 2). Fore femora with single short, retrolateral, apical spine and mid femora with a pair of prominent apical spines (one prolateral and one retrolateral). Hind femora with 5–13 inferior retrolateral spines (Table 3). Fore and mid tibiae each with four inferior linear spines (two prolateral and two retrolateral), three inferior apical spines (one prolateral, one ventral and one retrolateral) and with one superior apical spine on fore tibiae (retrolateral) and two superior apical spines on mid tibiae (one prolateral and one retrolateral). Hind tibiae with variable number of linear, short, fixed spines on superior surface (in two rows of prolateral and retrolateral) (Table 3), and typical eight, articulated apical spines. The longest pair of these (sometimes referred to as spurs) are shorter than 1st and/or 2nd segments of hind tarsi combined. The 1st tarsal segment of hind tibiae have between one to four superior spines.

COLOURATION. Head with short brown or black hairs. Face white and glabrous always with 4 vertical black stripes below eyes and inner sides of antennal scapes, and a pair of lateral marks on clypeus (Fig. 8A). Body usually black with small brown or yellowish spots on thoracic (dorsolateral) and abdominal tergites, covered in black and brown short hairs. Legs dark and pale speckled, hind femur dark to pale speckled especially at proximal half.

Table 2. Length of body elements of adult *Diestramima* type specimens from Bhutan (mm). Abbreviations and method of measuring applied are provided in table 1.

Trait	Sex	<i>D. matarmagna</i> sp. nov. 2 males 2 females	<i>D. minjiwoonga</i> sp. nov. 1 male	<i>D. multidenticuli</i> sp. nov. 2 males 2 females	<i>D. phubdorjia</i> sp. nov. 2 males 1 female	<i>D. samkhara</i> sp. nov. 1 male 2 females	<i>D. tsongkhapa</i> 2 males 2 females
BL	Male	11.9-16	12.6	15.6-16.2	10.4-12.7	12	18.2-20.9
	Female	13.8-18.5	-	13.3-15	12.9	12.6-13.2	13.1-15.1
PL	Male	4.7-5.2	5.1	5.2-5.5	4.4	4.5	5.3-6.5
	Female	5.8	-	4.7-5.6	4.8	3.2-5.5	5.7-6
FFL	Male	9.5	13.1	10-10.3	10.5	9.7	11.8-15.5
	Female	13.4-13.8	-	8.1-9.7	11.5	7.6-12.6	11.7-13.3
FTL	Male	9.6-10.8	12.5	10.3-10.4	11.2-11.3	10.1	12.3-15.8
	Female	13.8-13.9	-	7.4-10.2	12.1	7.7-11.5	12.1-14.6
FBTL	Male	2.9-3.5	4.4	3.1-4.1	3.9	3.5	4.7-5.4
	Female	4.3-4.8	-	3.3	4.1	2.7-4.2	4.2-4.8
MFL	Male	8.2-10.2	10	8.8-9.3	8.4-9.5	8	9.9-13
	Female	11.1-11.6	-	7.3-8.7	10	5.9-9.2	10.1-11.6
MTL	Male	7.7-11	10.3	8.4-9.4	9.1-10.1	8.9	11-13.9
	Female	11.5-12.7	-	7.3-8.9	10.3	6.6-11	11-14.5
MBTL	Male	2.4-2.7	3.2	2.7-2.9	2.1-3	2.7	3.4-3.7
	Female	3.2-3.4	-	2.2-2.3	2.9	2-2.4	3.4 -3.9
HFL	Male	18.2-21.2	21.5	20.4	18.9-19.1	18.2	23.6-29.7
	Female	23.5-24.4	-	17.1-20.6	21.4	13.6-22.3	19.2-22.6
HTL	Male	19.4-22.3	23	22-22.4	21.2-21.8	20.3	30.1-32.2
	Female	27-27.1	-	18-22.5	24.6	14.7-23.8	22.2-25.7
HBTL	Male	3.9-4.2	4.6	4-4.4	4.3-4.6	4	5.3-5.6
	Female	4.3-5.5	-	3.5-4	4.1	2.8-4.5	4.5-6.1
Eye W/L	Male	0.8/1.2-1.4	1-1.1/ 1.4-1.7	0.7-0.8/1-1.2	0.9/1.4	0.7/1.3	1-1.1/ 1.4-1.7
	Female	0.8-0.9/1.5-1.6	-	0.8-0.9/1.3-1.4	1.3/0.7	0.6-1/1-1.7	0.9/1.7-1.8
MPL	Male	2.5-4.2/ 3.2-4.9/ 4.5-6.6	11 (3, 3.1, 4.8)	3/3.5/49- 5.1	2.6-2.7/ 3.2-3.4/ 3.6-4.7	(2.7, 2.5, 3.9)	3.8-4.3/ 3.9-4.4/ 5.4-6.3
	Female	3.4-3.9/3.8-4.3/ 5.8-6	-	2.9-3.1/ 3.1/ 5-5.2	2.7/ 3.6/ 3.6	2-3.4/ 3.5-4.9/ 4.7-6.6	3.2-4.5/3.4-5.2/3.6-7.6
Sgp L	Male	1.2-1.6/0.7-1	1.2/1.1	2-2.1/1.5-1.6	1.6-2/1.3-1.4	1.2/0.9	2.8/1.5
	Female	0.5	-	1.4/0.7	1.7/1.4	-	1.4/ 1.6
CL	Male	3.7-4.2	4.8	4.1	3.1-3.2	2.5	4.5
	Female	4.8-5.2	-	2.3-4.2	3.4	2.3-4.5	5.7
OvL	Female	10.8-17.2	-	6.8-17.1	15	5.7-8.6	15.8-22.5

Table 3. Number of spines on hind leg of *Diestramima* type specimens from Bhutan. The number of spines and arrangement on the fore and mid legs are as per the generic description (supplementary table S1). Where left and right legs differ in the number of spines the count from the second limb is provided in parenthesis.

Leg element	Spines	<i>D. matermagna</i> sp. nov.		<i>D. minjiwoonga</i> sp. nov.		<i>D. multidenticuli</i> sp. nov.		<i>D. phubdorjia</i> sp. nov.		<i>D. samkhara</i> sp. nov.		<i>D. tsongkhapa</i>	
		1 male	1 female	1 male		1 male	1 female	1 male	1 female	1 male	1 female	1 male	1 female
Hind femur	Inferior (retro)	9	9	13		7	5	6	8	11	11	8	7
Hind Tibia	Superior linear (pro)	41	44(41)	38 (36)		55(53)	49	30(32)	36(38)	36	36	45(40)	45(42)
	Superior linear (retro)	37(36)	37(38)	39(37)		54(49)	52	26(29)	34(38)	35	35	46	46(43)
	Superior subapical (pro, retro)	1,1	1,1	1,1		1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1
	Apical spines	6	6	6		6	6	6	6	6	6	6	6
Hind basitarsus	Superior spines on 1st tarsus	3	4	4		2	2	1	2	3	2	3	4

***Diestramima tsongkhapa* (Würmli, 1973)**

(Figure 6A–K)

Thirteen specimens in our collections were identified as *D. tsongkhapa*. These were collected from four sites in central Bhutan, increasing the known distribution of this species (Fig. 4). Here we present images and summary of the key traits of this species (Fig. 6).

Material examined. BHUTAN • 5 adult females; Philligang, Nabji, Korphoog, Trongsa; 27.178°N, 90.535°E; 1381 m asl; 30 Jul. 2022; Sanjit Rai leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5516, MPN_CW5520, MPN_CW5517, MPN_CW5518, MPN_CW5519. • 4 adult males; Philligangju, Nabji, Korphoog, Trongsa; 27.180°N, 90.535°E; 1305 m asl; 30 Jul. 2022; Sanjit Rai leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5522, MPN_CW5521, MPN_CW5523, MPN_CW5524. • 2 male and 2 female adults; Namkye, Nabji, Korphoog, Trongsa; 27.165°N, 90.562°E; 2252; m asl; 22 Jul. 2022; Sanjit Rai leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5525, MPN_CW5526, MPN_CW5527, MPN_CW5528.

Male. Body medium in size (~ 20 mm). Median ocellus oval and lateral ocelli circular situated on lateral surface of basal fastigium vertici. Posteromedian projection of 7th abdominal tergite rather high in profile and long (Fig. 6E), obviously surpassing apices of paraproct, gradually narrowing towards apex when viewed dorsally, apex slightly extended with distinct notch (Fig. 6D). Paraproct with broad proximal half tapering abruptly and curved upward, apex obtuse (Fig. 6C & D). Subgenital plate large, curved, posterior edge slightly crimped, apex rounded (Fig. 6C). Cerci slender, conical, apices almost acute. Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Head dorsally brown with abundant short brown hair. Body black with many small brown or yellowish spots on thoracic and abdominal tergites, covered in black and brown short hairs. A pale, thin median line present but inconspicuous (Fig. 6F). Paraproct black when viewed dorsally. Cerci white with black spots on dorsal side with short yellowish-brown hair and longer hair.

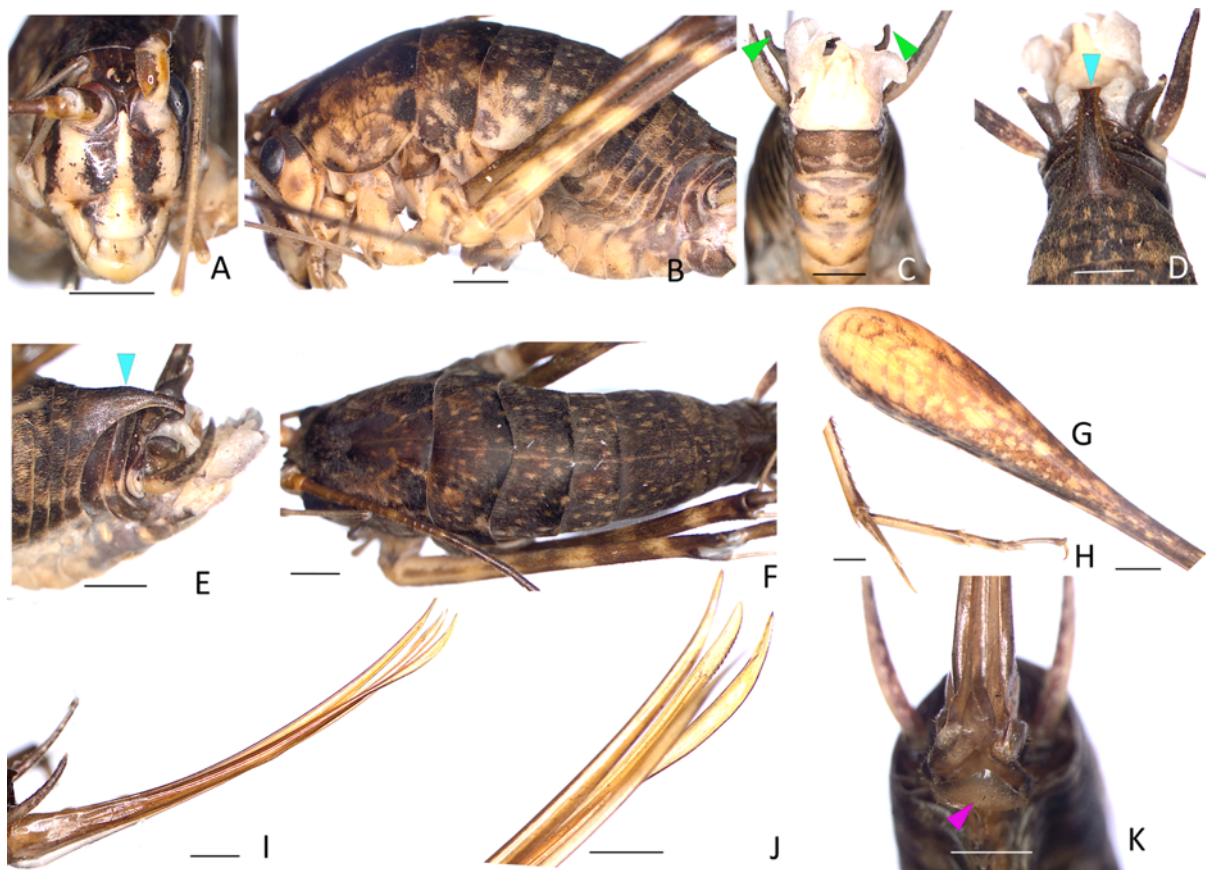


Figure 6. *Diestramima tsongkhapa*. Male: (A) Anterior view of head. (B) Lateral view of body. (C) Ventral view of lower abdomen showing finger-like distal part of paraproct (green arrows). (D) Dorsal view of lower abdomen showing notched apex of 7th abdominal tergite (blue arrow). (E) Lateral view of lower abdomen showing high profile 7th abdominal tergite (blue arrow). (F) Dorsal view abdomen. (G) Lateral view of hind femur and (H) basitarsus. Female: (I) Lateral view of ovipositor and (J) crenulated apex on lower valves. (K) Ventral view of lower abdomen showing subgenital plate (pink arrow). Scale bars = 2 mm.

Female. The general appearance is similar to male. Posteromedian process of 7th abdominal tergite short and angular with posteromedial apex obtusely rounded completely covering 8th abdominal tergite, 9th tergite broader and more rounded. Subgenital plate almost translucent, simple, triangular, apex obtusely rounded, slightly curved upward (Fig. 6K). Ovipositor slender and longer than body, slightly curved, apices acute, distal half of lower valves crenulated, upper valves smooth (without hair along the margins) (Fig. 6I & J). Size of body elements (Table 2) and combination of leg spines (Table 3) are similar to male.

***Diestramima matermagna* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 7A–K)

Holotype. BHUTAN • 1 adult male; Guru Drakphu Nye, Wangphu, Gomdar, Samdrup Jongkhar; 26.982°N, 91.584°E; 1028 m asl; 29 May 2022; C. Dorji & Jigme leg.; Subtropical forest: limestone cave entrance; MPN_CW5486; NBCB146.

Paratype. BHUTAN • 1 adult female; Guru Drakphu Nye, Wangphu, Gomdar, Samdrup Jongkhar; 26.982°N, 91.584°E; 1028 m asl; 29 May 2022; C. Dorji & Jigme leg.; Subtropical forest: limestone cave entrance; MPN_CW5487; NBCB152.

Other material. BHUTAN • 2 adult females; Guru Drakphu Nye, Wangphu, Gomdar, Samdrup Jongkhar; 26.982°N, 91.584°E; 1028 m asl; 29 May 2022; C. Dorji & Jigme leg.; Subtropical forest: limestone cave entrance; MPN_CW5485, MPN_CW5488. • 1 adult male; Yurung bridge, Khar, Pema Gatshel, 27.040°N, 91.380°E; 571 m asl; 31 May 2022; C. Dorji, Tshewang Namgay & Jigme leg.; Subtropical forest: cliff base under leaf litter and small rocks; MPN_CW5489. • 6 male and 5 female adults; Marung bridge, Khar, Pema Gatshel; 27.011°N, 91.420°E; 923 m asl; 25 May 2022, 31 May 2022; C. Dorji, Tshewang Namgay & Jigme leg.; Subtropical forest: cliff base under leaf litter and small rocks; MPN_CW5479, MPN_CW5480, MPN_CW5481, MPN_CW5482, MPN_CW5536, MPN_CW5537, MPN_CW5538, MPN_CW5539, MPN_CW5540, MPN_CW5490, MPN_CW5491. • 3 male and 5 female adults; Khelaphung, Kharungla, Brekha, Trashigang; 27.155°N, 91.628°E; 2039 m asl; 1 Jul. 2022; C. Dorji & Jigme leg.; Cool broadleaf forest: surrounding huge boulders and nearby vegetation; MPN_CW5492; MPN_CW5493, MPN_CW5494, MPN_CW5495, MPN_CW5496, MPN_CW5497, MPN_CW5498, MPN_CW5499.

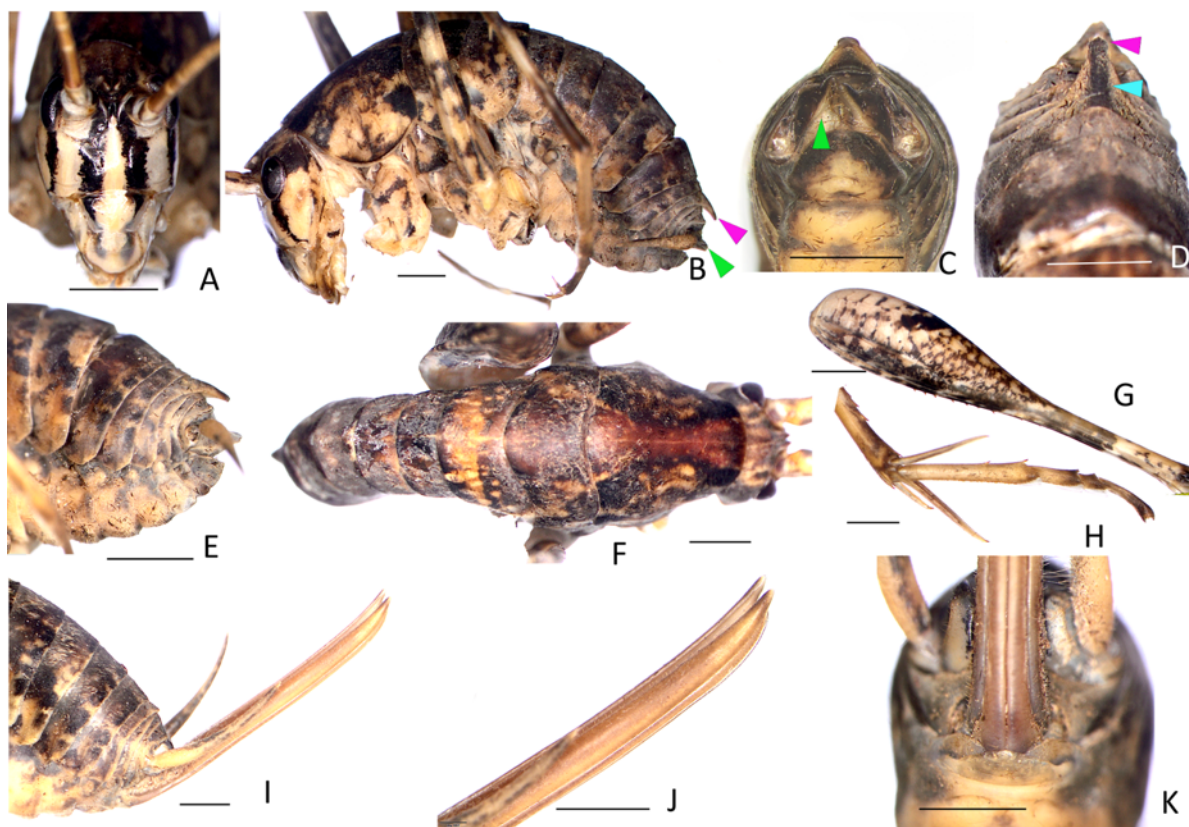


Figure 7. *Diestramima matermagna* sp. nov. Male holotype (MPN_CW5486; NBCB146): (A) Anterior view of head. (B) Lateral view of body showing short 7th abdominal tergite (pink arrow) and paraproct (green arrow). (C) Ventral view of lower abdomen showing short triangular paraproct (green arrow). (D) Dorsal view of lower abdomen showing narrowing 7th abdominal tergite (blue arrow) and truncate or slightly convex apex (pink arrow). (E) Lateral view of lower abdomen. (F) Dorsal view of body. (G) Lateral view of hind femur and (H) basitarsus. Female (MPN_CW5487; NBCB152): (I) Lateral view of ovipositor and (J) finely serrated apex. (K) Ventral view of lower abdomen showing subgenital plate. Scale bars = 2 mm.

Description. Male. Body small in size (<17 mm). Median ocellus oblong and lateral ocelli circular. Posteromedian projection of 7th abdominal tergite very short never extending beyond tenth abdominal tergite, gradually narrowing when viewed from above (Fig. 7B & D), truncate or slightly convex apex (Fig. 7D). Paraproct rather short and almost triangular, apices acute (Fig. 7B & C). Cerci conical, apices acute. Subgenital plate similar to *D. tsongkhapa*. Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Female. General appearance is similar to male but larger (body >14 mm long, hind legs 7 mm longer than male's; Table 2). Posteromedian projection of 7th abdominal tergite short and angular with posteromedial apex obtusely rounded covering 8th abdominal tergite. Ovipositor

narrow and almost straight (slightly curved upward at distal half), and as long as body (Fig. 7I). Lower valves are finely serrated at distal end, with acute apices (Fig. 7J). Upper valves with equally spaced short hair along dorsal margins (almost starting from the base) (Fig. 7J), gradually narrowing with slightly acute apex (Fig. 7I). Subgenital plate broad and triangular with obtusely angular apical part (Fig. 7K). Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Colouration. Head dorsally brown with abundant short brown-yellow hairs. Body brown with few small yellowish spots along posterior edge thoracic and abdominal tergites, covered in black and brown short hairs. Paraproct black dorsally, and white ventrally. Cerci white with brown spots on dorsal side. The distal half of cerci are covered by short yellowish-brown hair and fewer longer hairs at proximal and mid areas.

Etymology. “*Matermagna*” is Latin for ‘great mother’—because adult females have larger bodies and longer hind legs than males.

***Diestramima minjiwoonga* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 8A–H)

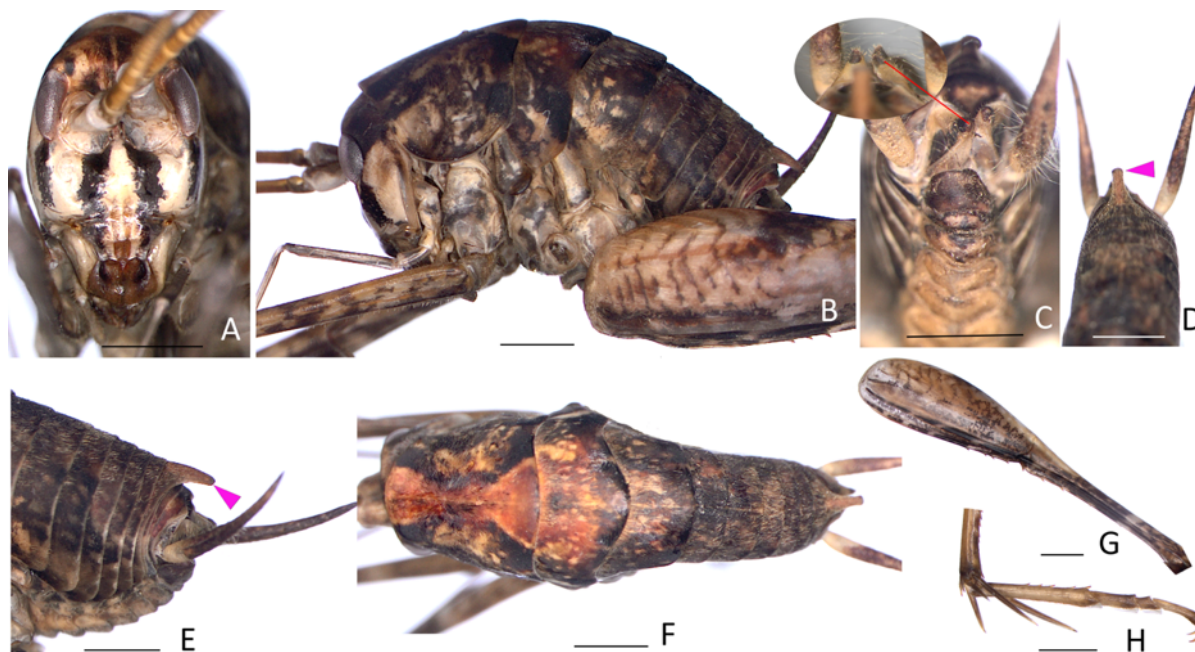


Figure 8. *Diestramima minjiwoonga* sp. nov. Male holotype (MPN_CW5484; NBCB147): (A) Anterior view of head. (B) Lateral view of body. (C) Ventral view of lower abdomen showing characteristic black spots on dorsal side of paraprocts. (D) Dorsal and (E) lateral view of lower abdomen showing 7th abdominal tergite (pink arrow). (F) Dorsal view of body. (G) Lateral view of hind femur and (H) basitarsus. Scale bars = 2 mm.

Holotype. BHUTAN • 1 adult male; Khandro Yeshi Tshogyel Nye, Minjiwoong peg, Serthi, Samdrup Jongkhar; 26.988°N, 92.012°E; 833 m asl; 28 May 2022; C. Dorji, Tshering Dorji & Jigme leg.; Subtropical forest: cave entrance; MPN_CW5484; NBCB147.

Description. Male. Body medium in size (~12 mm). Median ocellus oval, and lateral ocelli almost circular situated on lateral surface of basal fastigium vertici. Posteromedian projection of 7th abdominal tergite is short covering only distal half of paraproct with lateral sides nearly parallel (Fig. 8E), lateral and apical margins slightly curved inwards with truncate apex. Paraproct rather short and triangular on lateral view, apices acute. Cerci slender, conical, apices acute. Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Colouration. Head dorsally black with abundant short brown-black hairs. Body black with small brown or yellowish spots on thoracic (dorsolateral) and abdominal tergites, covered in black and brown hairs. A pale, thin median line is present but inconspicuous (Fig. 8F). Paraproct white with black spots at the distal end when viewed dorsally (Fig. 8C). Cerci with two-thirds end black with dense short yellowish-brown hairs and fewer longer hairs in the mid region.

Female. Unknown.

Etymology. The new species is named for the type locality Minjiwoong under Serthi geog, Samdrup Jongkhar district.

***Diestramima multidenticuli* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 9A–K)

Holotype. BHUTAN • 1 adult male; Bawoong, Ramjar, Trashy Yangtse; 27.416°N 91.586°E; 1582 m asl; 2 Jul. 2022; C. Dorji & Jigme leg.; Temperate forest: under boulders and surrounding vegetation; MPN_CW5500; NBCB148.

Paratype. BHUTAN • 1 adult female; Bawoong, Ramjar, Trashy Yangtse, 27.416°N, 91.586°E; 1582 m asl; 2 Jul. 2022; C. Dorji & Jigme leg.; Temperate forest: under boulders and surrounding vegetation; MPN_CW5534; NBC153.

Other material. BHUTAN • 3 male and 5 female adults; Bawoong, Ramjar, Trashy Yangtse; 27.416°N 91.586°E; 1582 m asl; 2 Jul. 2022; C. Dorji & Jigme leg.; Temperate forest: under

boulders and surrounding vegetation; MPN_CW5501, MPN_CW5502, MPN_CW5503, MPN_CW5504, MPN_CW5531, MPN_CW5532, MPN_CW5533, MPN_CW5535.

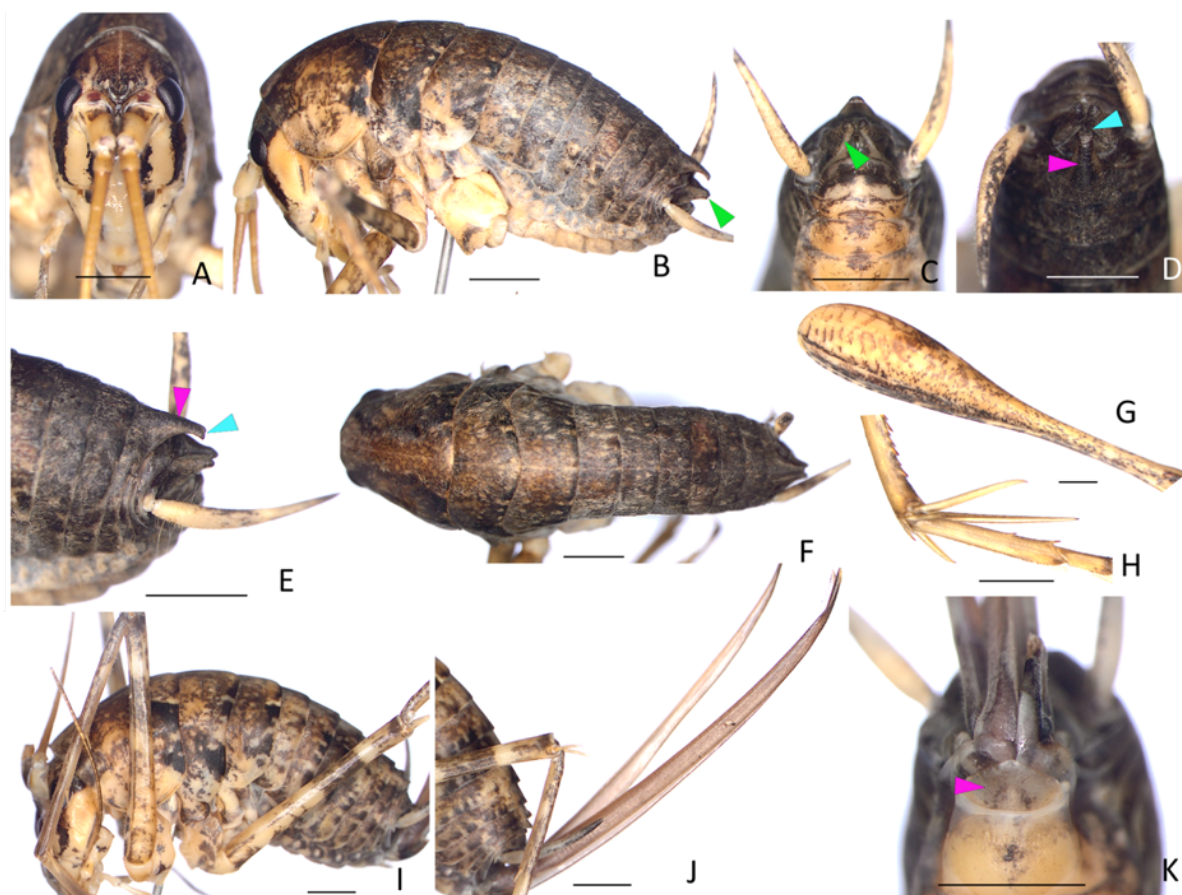


Figure 9. *Diestramima multidenticuli* sp. nov. Male holotype (MPN_CW5500; NBCB148): (A) Anterior view of head. (B) Lateral view of body showing short paraproct (green arrow). (C) Ventral view of lower abdomen showing short triangular paraproct (green arrow). (D) Dorsal and (E) lateral view of lower abdomen showing short narrowing 7th abdominal tergite (pink arrow) and truncated apex (blue arrow). (F) Dorsal view body. (G) Lateral view of hind femur and (H) basitarsus. Female (MPN_CW5534; NBC153): (I) Lateral view of abdomen. (J) Lateral view of ovipositor. (K) Ventral view of lower abdomen showing subgenital plate (pink arrow). Scale bars = 2 mm.

Description. Male. Body medium in size (~16 mm). Median ocellus oval and lateral ocelli circular situated on lateral surface of basal fastigium verticis. Posteromedian projection of 7th abdominal tergite short covering only two-thirds of paraproct (Fig. 9B & E), narrowing to apex (Fig. 9D), lateral margins not curved inwards, apex truncate with slight downward bend (Fig. 9E). Paraproct rather short and triangular on lateral view, apices acute (Fig. 9C & E). Cerci conical, apices acute. This species is morphologically similar to *D. tsongkhapa* but differs by its smaller body (15.6–16.2 mm), shorter 7th abdominal tergite with truncated apex, and

relatively shorter paraproct. This species is not phylogenetically sister to *D. tsongkhapa* (Fig. 6). Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Colouration. Head dorsally brown covered by brown and black hairs. A pale, thin median line present but inconspicuous (Fig. 9F). Rostral tubercles black from above and white on lateral sides and apices. Body brown with small brown spots along posterior edges of thoracic abdominal tergites, covered in black and brown short hairs. Paraproct black when viewed dorsally. Cerci white with black colouration on dorsal part with short yellowish-brown hairs and fewer longer at proximal half.

Female. Body colour much lighter than males with few larger dark black spots along posterior edge of thoracic and abdominal tergites (Fig. 9I). Same body size and leg length as male (Table 2). Posteromedian projection of 7th abdominal tergite short and angular with posteromedial apex obtusely rounded completely covering 8th and 9th abdominal tergite. Subgenital plate membranous, broad and short, almost triangular, apex obtusely rounded, slightly curved upward (Fig. 9K). Ovipositor narrow and as long as body (Fig. 9J). Combination of leg spines is similar to males.

Etymology. The name refers to the very large number of tiny linear spines on the superior surface of the hind tibiae.

***Diestramima phubdorjia* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 10A–H and Figure 12A–F)

Holotype. BHUTAN • 1 adult male; Tshangkha, Tangsibji, Trongsa; 27.440°N, 90.420°E; 2161 m asl; 6 Jul. 2022; C. Dorji, Karma Chorten & Jigme leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5509; NBCB149.

Paratype. BHUTAN • 1 adult female; Tshangkha, Tangsibji, Trongsa; 27.440°N, 90.420°E; 2161 m asl; 6 Jul. 2022; C. Dorji, Karma Chorten & Jigme leg.; Cool broadleaf forest: under fallen tree branches and **leaf** litter; MPN_CW5510; NBCB154.

Other material. BHUTAN • 1 adult male; Tshangkha, Tangsibji, Trongsa; 27.440°N, 90.420°E; 2161 m asl; 6 Jul. 2022; C. Dorji, Karma Chorten & Jigme leg.; Cool broadleaf forest: under fallen tree branches and leaf litter.; MPN_CW5508. • 1 adult female; Philligang, Nabji,

Korphoog, Trongsa; 27.178°N, 90.535°E; 1381 m asl; 30 Jul. 2022; Sanjit Rai leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5515.

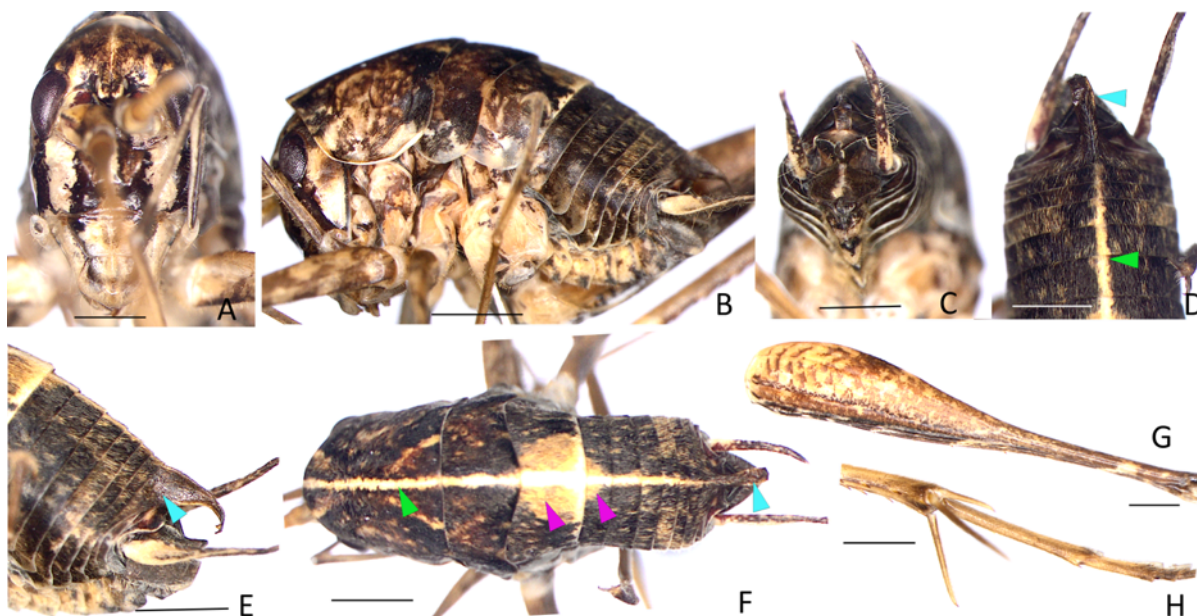


Figure 10. *Diestramima phubdorjia* sp. nov. Male holotype (MPN_CW5509; NBCB149): (A) Anterior view of head. (B) Lateral view of body. (C) Ventral view of lower abdomen. (D) Dorsal view of lower abdomen showing flat and parallel distal half of 7th abdominal tergites (blue arrow) and dorsal pale medial line on abdomen (green arrow). (E) Lateral view of lower abdomen showing lateral curving (blue arrow) and dichotomous apex of 7th abdominal tergite. (F) Dorsal view of body, showing pale median line (green arrow), dorsal median white spot on metanotum and 1st tergite (pink arrows), and long 7th abdominal tergite projection (blue arrow). (G) Lateral view of hind femur and (H) basitarsus. Scale bars = 2 mm.

Description. Male. Body small (10–13 mm). Median ocellus oval and lateral ocelli circular situated on lateral surface of basal fastigium verticis. Posteromedian projection of 7th abdominal tergite long, obviously surpassing apices of paraproct (Fig. 10E & F; Fig. 11A). The first half concaved laterally from the sides (Fig. 10E; Fig. 11A), second half flat and parallel curving downwards. The apex slightly curved inwards or downward (Fig. 10E; Fig. 11C), apical area of posteromedian projection dichotomous and acute when viewed from ventral side (Fig. 10C & E; Fig. 11C). Paraproct rather long (not as long as *D. tsongkhapa*), proximal half broad, narrowing towards apex, obtuse, slightly curved from middle directing backward. Subgenital plate similar to sister species *D. tsongkhapa* (Fig. 6C). Cerci slender, conical, apices obtuse. Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Colouration. Head dorsally yellowish or white and black. The body is mostly very dark to black with or without small pale spots and numerous short black and yellow hairs. A pale dorsomedial stripe (thin or thin) extends from the pronotum to the 2nd or 6th abdominal tergite (Fig. 10F). White spots possible on metanotum and 1st tergite (Fig. 10F). Paraproct black. Cerci black with white on ventral and basal parts, and with dense short yellowish-brown hairs and few long pale hairs at the base (Fig. 10C).

Female. Body colour darker than male without white spots on metanotum and 1st abdominal tergite. Median line is rather thin extending only until 2nd abdominal tergite. Posteromedian projection of 7th abdominal tergite short and angular with posteromedial apex rounded completely covering 8th abdominal tergite (Fig. 11D). Subgenital plate almost translucent, broad and short, almost convex with obtusely rounded apex (Fig. 11F). Ovipositor slightly longer than body length, thin. Upper valves broad and apex acute. Lower valves with numerous fine teeth from below the apex (Fig. 11). Size of body elements (Table 2) and leg spine combination (Table 3) are similar to male.

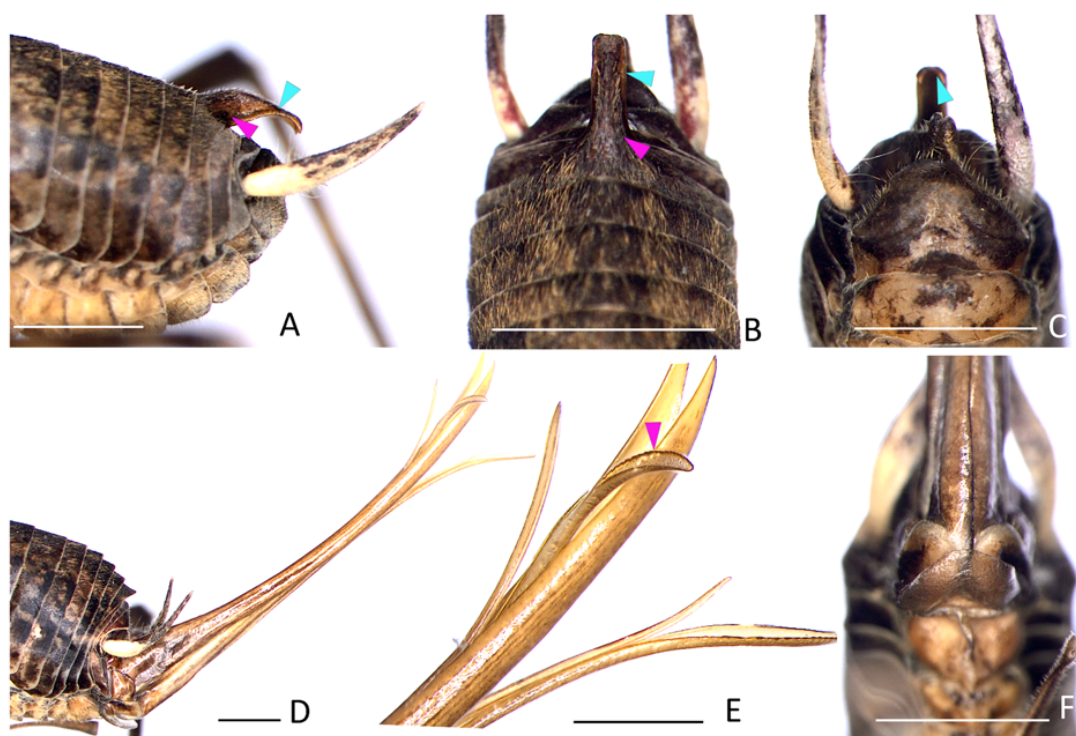


Figure 11. *Diestramima phubdorjia* sp. nov. Male (MPN_CW5508): (A) Lateral and (B) dorsal views of lower abdomen showing lateral curving (pink arrow) on proximal half and flat apex on distal half (blue arrow) of 7th abdominal tergite projection. (C) Ventral view of lower abdomen showing dichotomous apex (blue arrow). Female (MPN_CW5510; NBCB154): (D) Lateral view of lower abdomen. (E) Lateral view of ovipositor showing fine serration on lower valve (pink arrow). (F) Ventral view of subgenital plate. Scale bars = 2 mm.

Etymology. The new species is named in honour of late Dr. Phub Dorji, the first president of the College of Natural Resources, Royal University of Bhutan.

***Diestramima samkhara* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figures 12A–K)

Holotype. BHUTAN • 1 adult male; Samkhar bridge, Gelephu, Sarpang; 27.009°N, 90.581°E; 942 m asl; 22 May 2022; C. Dorji & Jigme leg.; Subtropical forest: under boulders above bridge; MPN_CW5541; NBCB150.

Paratype. BHUTAN • 1 adult female; Samkhar bridge, Gelephu, Sarpang; 27.009°N, 90.581°E; 942 m asl; 22 May 2022; C. Dorji & Jigme leg.; Subtropical forest: under boulders above bridge; MPN_CW5478; NBCB155.

Other material. BHUTAN • 2 adult females; Samkhar bridge, Gelephu, Sarpang; 27.009°N, 90.581°E; 942 m asl; 22 May 2022; C. Dorji & Jigme leg.; Subtropical forest: under boulders above bridge; MPN_CW5542, MPN_CW5543.

Description. Male. Body small (<14 mm). Median ocellus oblong and lateral ocelli circular. Posteromedian projection of 7th abdominal tergite short covering only proximal part of paraproct (Fig. 12B & E), shallowly grooved from dorsomedial until apex (Fig. 12C), barely wider, almost parallel lateral edges and truncate with small notch at median part of apex (Fig. 12C). Paraproct rather short and almost triangular on lateral view, apices acute (Fig. 12D & E). Cerci conical, apices acute, Subgenital plate similar to *D. tsongkhapa*. Linear measures of body elements (Table 2). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 3).

Colouration. Head dorsally brown covered by brown-yellow hairs. Rostral tubercles brown from above. Maxillary palps brown with white in mid region of each segment. Body brown with few small brown/white spots along posterior edges of thoracic and abdominal tergites, covered in black and brown short hairs. A pale, thin median line present but inconspicuous extending only up to 2nd or 3rd tergites (Fig. 12F). Paraproct black when viewed dorsally. Cerci white with black spots on dorsal side of distal half, and with short yellowish-brown hair and fewer longer hair at proximal half.

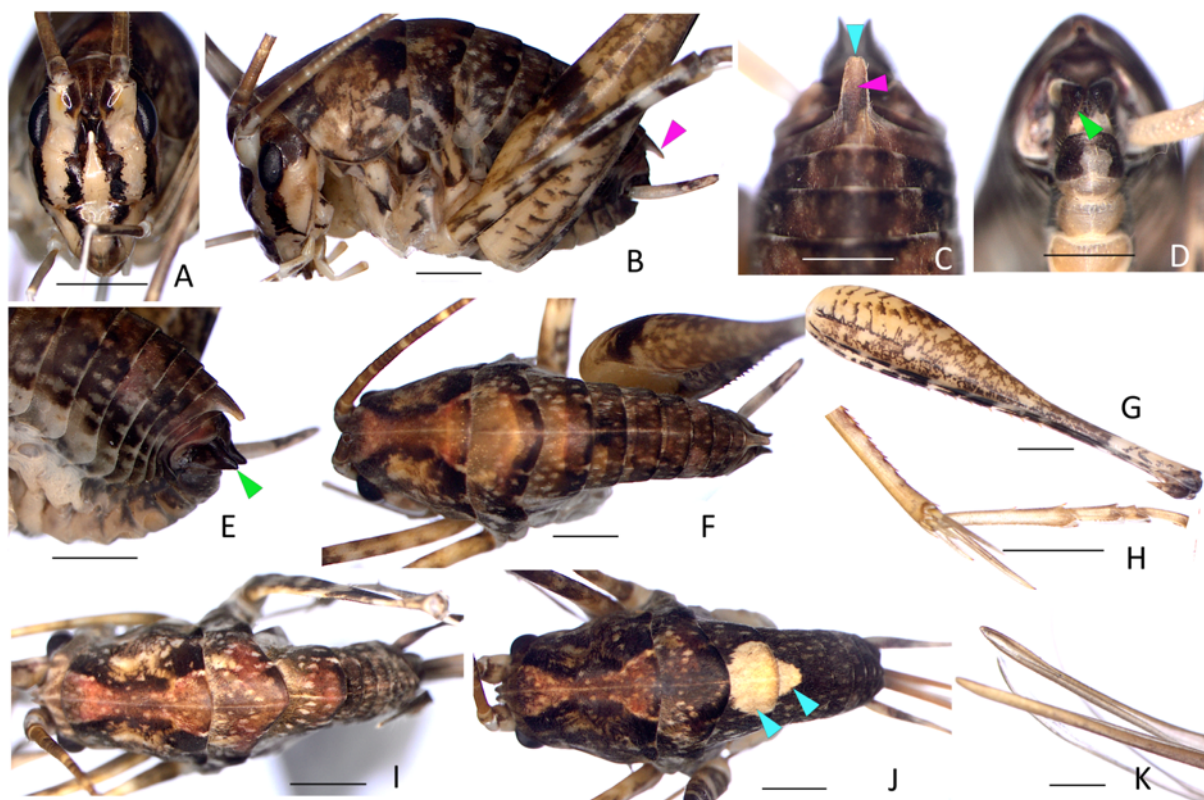


Figure 12. *Diestramima samkhara* sp. nov. Male holotype (MPN_CW5541; NBCB150): (A) Anterior view of head. (B) Lateral view of body showing short projection of 7th tergite (pink arrow). (C) Dorsal view of lower abdomen showing dorsal shallow groove (pink arrow) and little notched apex (blue arrow) on projection of 7th abdominal tergite. (D) Dorsal and (E) lateral views of lower abdomen showing short triangular paraproct (green arrow). (F) Dorsal view of body. (G) Lateral view of hind femur and (H) basitarsus. Female (MPN_CW5478; NBCB155): (I) Dorsal view of body. (J) Female colour variation: Dorsal view of body showing metanotum and 1st tergite dorsomedial white spots (blue arrows). (K) Lateral view of ovipositor. Scale bars = 2 mm.

Female. General appearance is similar to male, but body colour can be lighter with reddish brown on dorsal parts of thoracic tergites. White spots dorsomedial or along posterior edges of thoracic and abdominal tergites (Fig. 12J). Posteromedian projection of 7th abdominal tergite short and angular with posteromedial apex obtusely rounded completely covering 8th abdominal tergite. Ovipositor narrow and almost straight, and shorter than body, slightly curved at apex. Lower valves without serration, acute. Upper valves with hair along the margins, slightly acute at apex. The size of body elements and combination of leg spines are similar to male.

Etymology. The species is named after the type locality Samkhar, Sarpang district.

Subfamily **RHAPHIDOPHORINAE**Genus **RHAPHIDOPHORA** Serville, 1838

Type species *Rhaphidophora picea* Serville, 1838

The genus *Rhaphidophora* was defined in 1838 with a single female specimen of one species and now includes 129 species (Cigliano et al. 2025). The general characteristics of the genus are agreed (Gorochov, 1999, 2012) and here we summarise the appearance of *Rhaphidophora* species considering traits that are shared.

HEAD. Oval, smooth and shiny except for maxillary palps and antennae covered in dense short hair. Fastigium projecting forward with longitudinal furrow along midline on dorsal surface, partially dividing apical half into a pair of rostral tubercles. Eyes oval, slightly bigger than antennal scapi. Median ocellus situated in between ventral margins of antennal scapi and, lateral ocelli occupying basal two-thirds of lateral surface of rostral tubercles.

THORAX. Body medium size and with abundant fine black or brown hairs. Anterior margin of pronotum generally straight and posterior margin obtusely convex or nearly straight. The posterolateral margin of pronotum straight or concave while posterolateral margins of mesonotum and metanotum are slightly oblique or almost rounded.

LEGS. Legs moderately long (Table 4). Fore femora with single short, prolateral, apical spine and mid femora with a pair of prominent apical spines (one prolateral and one retrolateral). Hind femora unarmed but with apical inferior spine (retrolateral). Fore tibiae with three inferior linear spines (one prolateral and two retrolateral), and two apical spines (one prolateral and one retrolateral). Mid tibiae with four superior linear spines (two prolateral and two retrolateral) and five inferior linear spines (three prolateral and two retrolateral), and two apical spines on inferior and superior sides (one prolateral and one retrolateral). Hind tibiae with variable number of linear, short, fixed spines on superior surface (in two rows of prolateral and retrolateral) (Table 4), and typical eight, articulated apical spines. The longest pair of these (= spurs) are longer than 1st and/or 2nd segments of hind tarsi combined. The 1st tarsal segment of hind tibiae have variable number of superior spines.

MALE TERMINALIA. Adult male terminalia show significant differences among species and are widely used as a key morphological trait for diagnosing species within this genus. A description of terminalia is provided for each newly described species.

Abdominal tergites usually without distinct posterior projections. The epiproct is weakly specialised, broad always projecting behind body. The posterior margin may bear one to two sclerotised spines of variable size and shape. Genitalia membranous without any sclerotization. Cerci slender, conical and usually longer than epiproct, covered in both short and long hairs. Subgenital plate is broad and fairly similar. Styli variable from stout to somewhat long in short hairs.

COLOURATION. Head and pronotum dorsally dark brown, face and mouthparts brownish. Rostral tubercles usually dark brown. Compound eyes small and black, brown or sometimes with maculation of black and white. Maxillary palps usually pale or with black and white distinct band on each segment. Antennae longer than body mostly pale brown in colour. Body dark brown or yellowish brown with irregular white patches along the posterior of abdominal tergites from above and slightly yellowish brown from beneath. Legs are general yellowish with somewhat darker colouration in joints. Hind femur paler speckled at proximal end and darker to distal end. Cerci and styli either black or white or both.

NYMPHS. General appearance of the nymph is usually similar to adults but terminalia used for distinguishing species are not well developed.

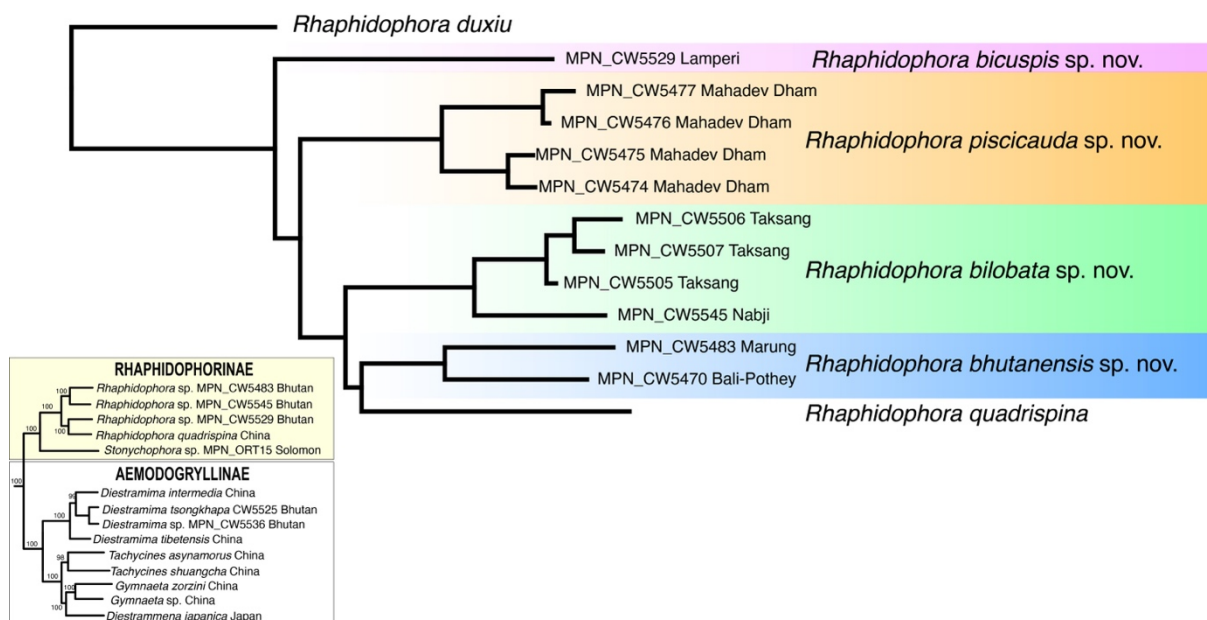


Figure 13. Neighbor-Joining (HKW) phylogeny of *Rhaphidophora* raphidophorids from Bhutan using 722bp alignment of mtDNA COI sequences. Inset: Phylogenetic relationships of genera within subfamily Rhaphidophorinae and Aemodogryllinae resulting from ML analysis of mtDNA sequences (COI) of 1455 bp.

Table 4. Length of body elements of *Rhaphidophora* type specimens from Bhutan (mm). Abbreviations and method of measuring each element provided in table 1.

Trait	<i>R. bhutanensis</i> sp. nov.	<i>R. bicuspis</i> sp. nov.	<i>R. bilobata</i> sp. nov.		
	1 male	1 male	1 male	1 female	1 male
BL	25.1	14.2	15.2	26.7	18.2
PL	7.8	3.1	4.8	6.5	5.8
FFL	9.1	5.4	6	7.3	6.3
FTL	9.8	5.9	6	6.9	6.2
FBTL	2.4	1.7	1.7	2	1.7
MFL	8.4	5.5	5.9	7.2	5.9
MTL	8.5	6.1	6.4	7.6	6.4
MBTL	2.1	1.4	1.5	1.3	1.2
HFL	24.2	12.4	15	19.1	15.9
HTL	21.9	12.1	12.3	16.1	14.9
HBTL	4.6	2.9	2.9	4.4	3.9
Eye W/L	1.2/1.6	0.8/1	0.9/1.3	1.1/1.7	0.8/1.4
MPL	2.9/3.5/4.5	1.7/1.8/2.7	2.3/2.4/3.6	3.5/2.6/3.3	2/ 2.2/ 3
Sgp W/L	3.2/1.3	2/0.7	2.8/0.6	1.6/0.6	2.8/0.9
SL	1.7	0.9	1.3	-	1
CL	8.1	6.4	4.3	6.4	5.1
OvL	-	-	-	14.1	-

Table 5. Number of spines on hind leg of type specimens from Bhutan (Genus *Rhaphidophora*). The number of spines and arrangement on the fore and mid legs are as per the generic description (supplementary table S2). When left and right legs differ in spine number the count from the second limb is provided in parenthesis.

Leg element	Spines	<i>R. bhutanensis</i> sp. nov.	<i>R. bicuspis</i> sp. nov.	<i>R. bilobata</i> sp. nov.		
		1 male	1 male	1 male	1 female	1 male
Hind femur	Inferior apical (retro)	1	0	0	0	0
Hind tibia	Superior linear (pro)	23(24)	21 (20)	22	23(20)	25 (22)
	Superior linear (retro)	23(23)	19 (20)	20	20(22)	22(23)
	Superior subapical (pro, retro)	1,1	1,1	1,1	1,1	1,1
	Apical	6	6	6	6	6
Basitarsus	Superior spines on 1 st tarsus	6	6	5	6	5

***Rhaphidophora bhutanensis* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 14A–G)

Holotype. BHUTAN • 1 adult male; Marung bridge, Khar, Pema Gatshel; 27.011°N, 91.420°E; 923 m asl; 25 May 2022; C. Dorji, Tshewang Namgay & Jigme leg.; Subtropical forest: cliff base under leaf litter and small rocks; MPN_CW5483; NBCB145.

Other material. BHUTAN • 1 nymph female; Bali-Pothey, Kilkhorthang, Tsirang; 27.464°N, 90.059°E; 1266 m asl; 1 May 2022; C. Dorji & Yeshi Phuntsho leg.; Temperate forest: under two huge boulders; MPN_CW5470.

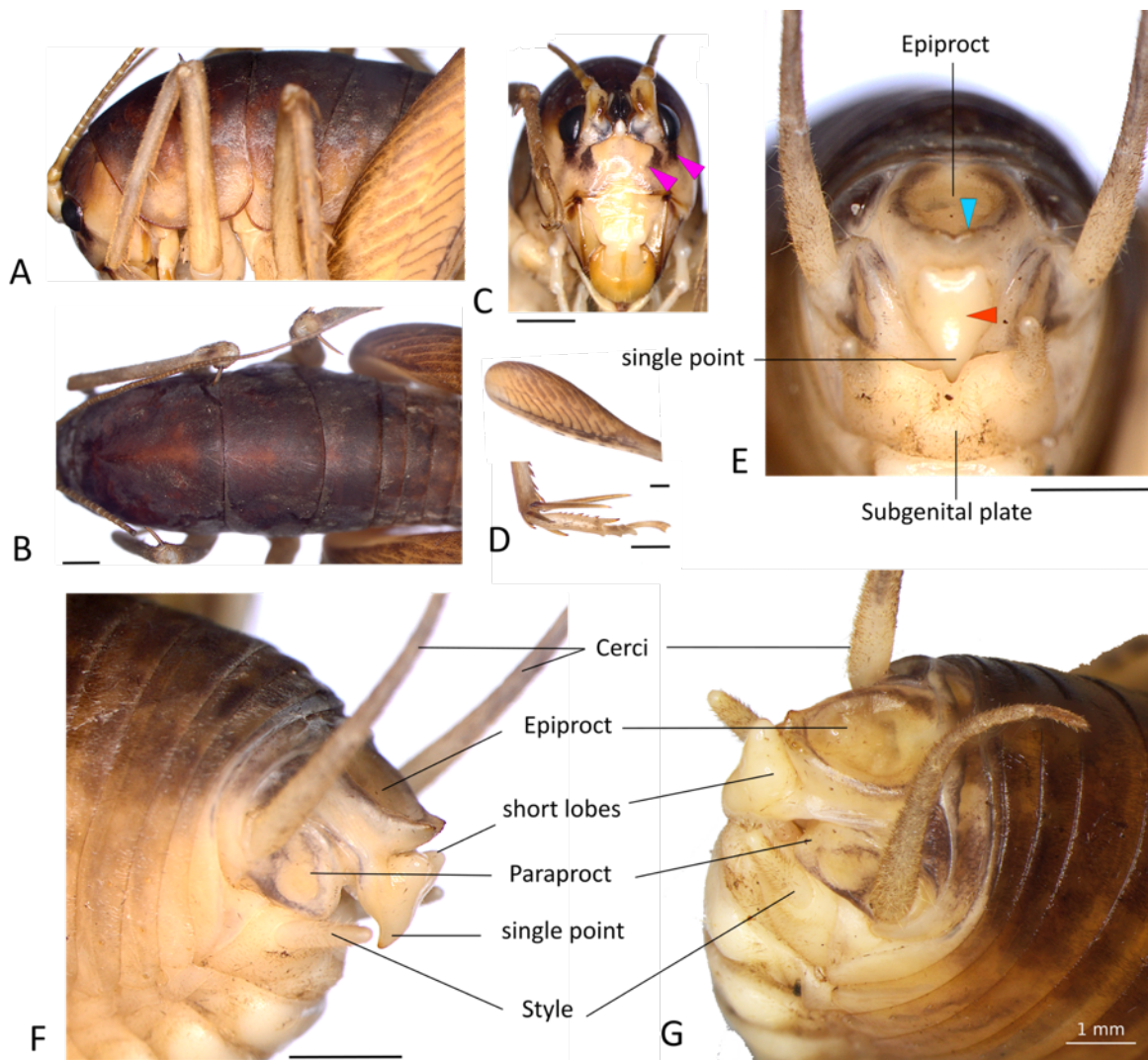


Figure 14. *Rhaphidophora bhutanensis* sp. nov. Male holotype (MPN_CW5483; NBCB145): (A) Lateral and (B) dorsal views of body. (C) Anterior view of head showing 4 vertical short black strips on the face. (D) Lateral view of hind femur and basitarsus. (E) Ventral view of lower abdomen showing epiproct projection (blue arrow) and ventral triangular basal plate of epiproct (red arrow). (F) Lateral and (G) latero-posterior views of lower abdomen. Scale bars = 2 mm.

Description. Male. Body medium in size (~25 mm). Median and lateral ocelli oval. Epiproct oval when viewed dorsally, not strongly curved but deeply grooved with a conspicuous pair of projections on the distal end directed slightly backwards (Fig. 14E). Ventral part of epiproct triangular, with a pair of large swollen lobes directed upward and triangular sclerotised apical lobe that usually remains inserted into genital cavity (Fig. 14E & F). Cerci almost twice as long as epiproct. Paraproct obtusely rounded (Fig. 14F). Subgenital plate with two ovoid lobes not completely divided (Fig. 14E). Styli stout (as short as the length of subgenital plate), more

cylindrical than conical (Fig. 14F & G). Linear measures of body elements (Table 4). Fore and mid leg spine details as per the generic description. Hind leg spine details (Table 5).

Colouration. Body brownish from above and yellowish brown from beneath. Legs light to medium brown. Head brownish dorsally, face yellowish with 4 short vertical black stripes on epicarnium below eyes and antennae scapi (Fig. 14C). Epiproct white/yellowish with light black lining along groove. Cerci and styli white with dense yellowish brown short hair and fewer longer hair along cerci.

Female. Unknown.

Etymology. The new species is named in recognition of the Bhutan nation.

***Rhaphidophora bicuspis* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 15A–G)

Holotype. BHUTAN • 1 adult male; Botanical Park, Lamperi, Thimphu; 27.498°N, 89.767°E; 2761 m asl; 22 Aug. 2022; C. Dorji leg.; Cool broadleaf forest: forest floor under leaf litter; MPN_CW5529; NBCB144.

Description. Male. Body small in size (<15 mm). Median and lateral ocelli almost oval and same in size. Lateral surface of a pronotum with central dark brown spots but inconspicuous (oval) (Fig. 15A). All abdominal tergites without distinct posterior projections. Posterior margin of tenth abdominal tergite concave. Epiproct simple, broad, oval, narrowing towards apex with carinated margin bearing a pair of sclerotised ventral spines (Fig. 15F & G). Ventral basal plate of epiproct triangular, forming a large single sclerotised apical spine (Fig. 15E). Cerci slender, conical and three times longer than epiproct. Styli stout and cylindrical with obtuse apex. Linear measures of body elements (Table 4). Fore and mid leg spine details as per the generic description except mid tibiae with two linear superior spines (one prolateral and one retrolateral). Hind leg spine details (Table 5).

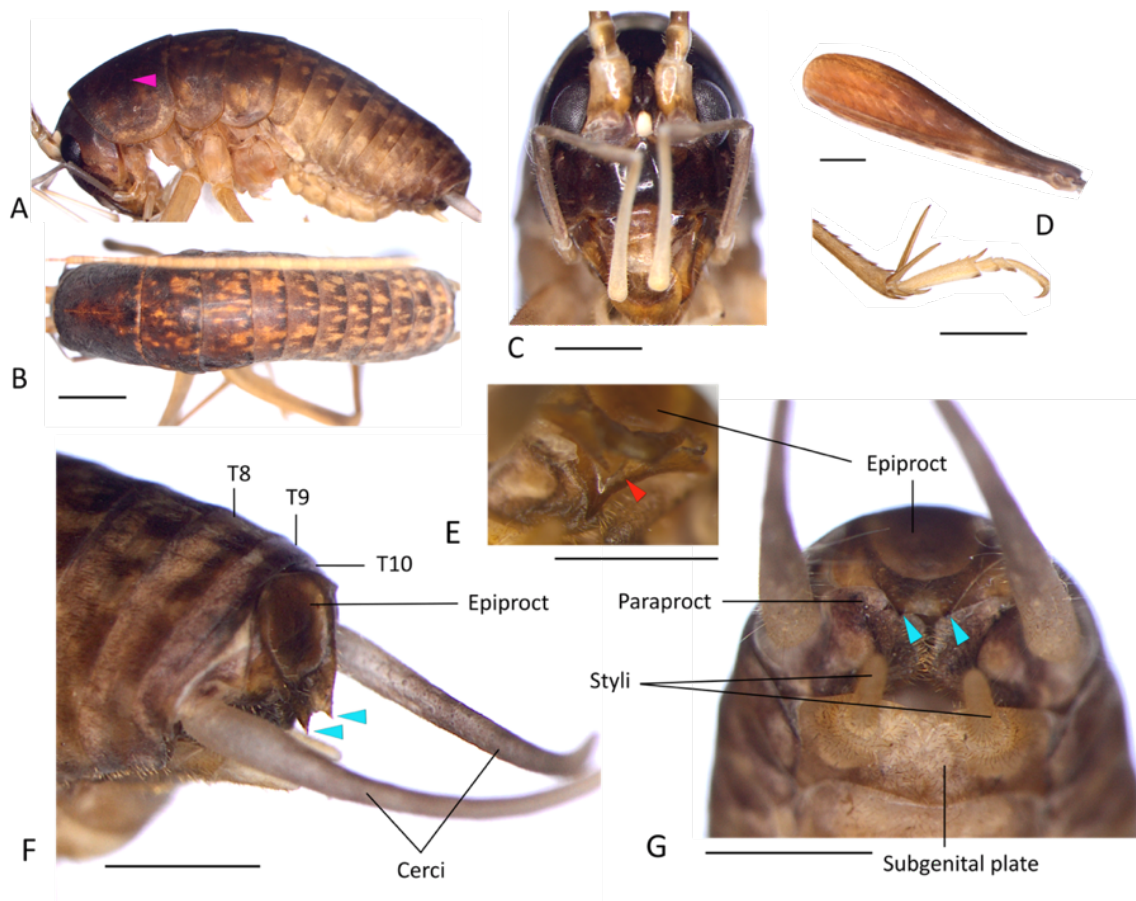


Figure 15. *Raphidophora bicuspis* sp. nov. Male holotype (MPN_CW5529; NBCB144): (A) Lateral and (B) dorsal views of body showing pronotal brown spot (pink arrow). (C) Anterior view of head. (D) Lateral view of hind femur and basitarsus. (E) Ventral view of epiproct showing triangular ventral basal plate with triangular sclerotised apical spine (red arrow). (F) Lateral and (G) ventral views lower abdomen showing a pair of sclerotised points (cusps) on distal margin of epiproct (blue arrows). Scale bars = 2 mm.

Colouration. Body yellowish brown with dark brown irregular patches along the posterior of abdominal tergites dorsally (Fig. 15A & B)) and slightly yellowish brown from beneath. Cerci and styli with dense yellowish brown short hair and fewer longer hair along the cerci.

Female. Unknown.

Etymology. “*Bicuspis*” is Latin for ‘two sides’ or ‘two cusps’ –derived from the paired spines on the ventral margin of male epiproct.

***Raphidophora bilobata* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 16A–K)

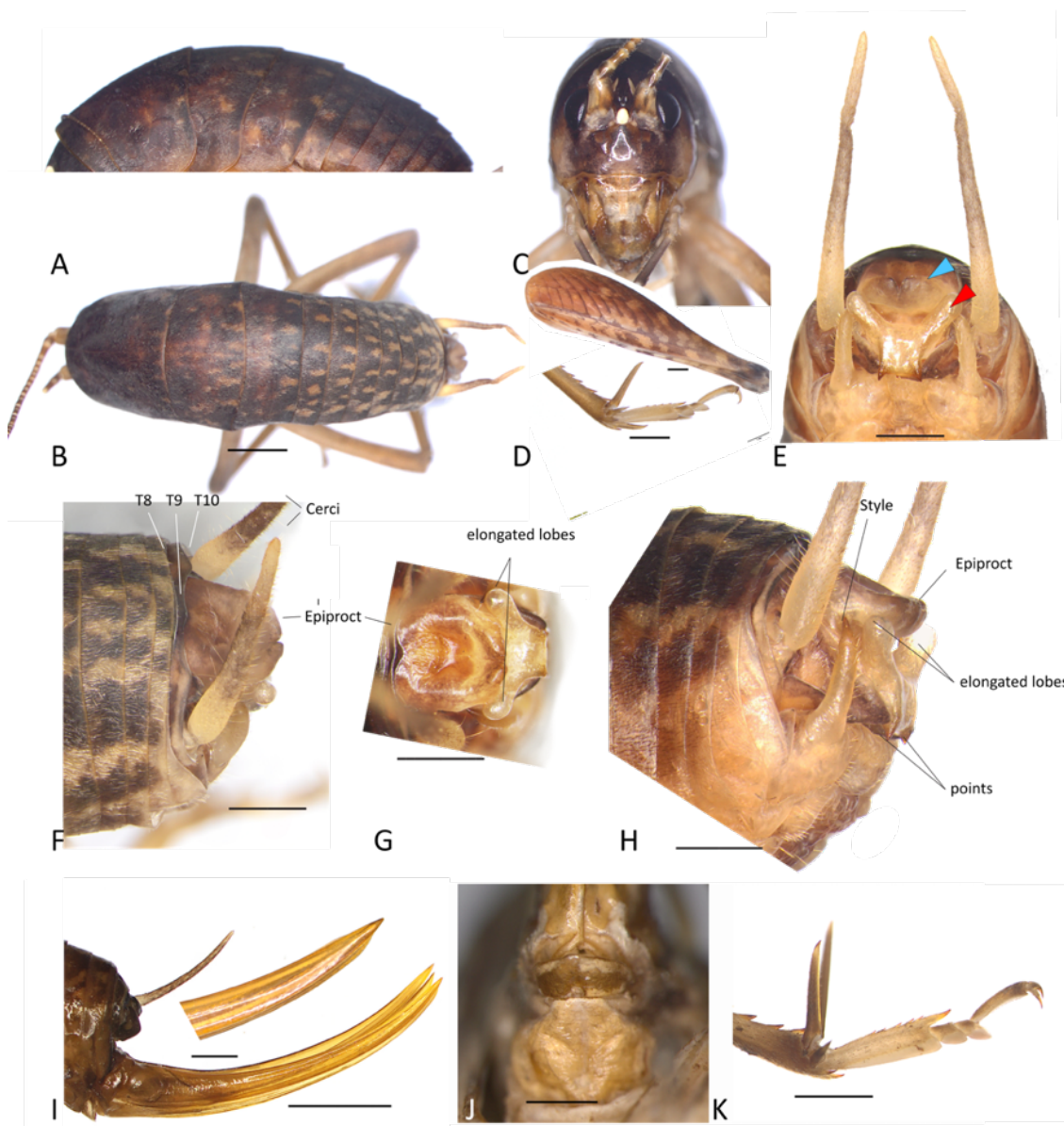


Figure 16. *Rhaphidophora bilobata* sp. nov. Male holotype (MPN_CW5505; NBCB142): (A) Lateral and (B) dorsal views of body. (C) Anterior view of head. (D) Lateral view of hind femur and basitarsus. (E) Ventral view of lower abdomen showing a pair of distal projections of epiproct (blue arrow) and elongated ventral basal plate lobes of epiproct (red arrow). (F) Dorsal view of lower abdomen. (G) Dorsal part of epiproct. (H) Lateral view of lower abdomen. Female (MPN_CW5544; NBCB151): (I) Lateral view of ovipositor. (J) Ventral view of subgenital plate. (K) Lateral view of hind basitarsus. Scale bars = 2 mm.

Holotype. BHUTAN • 1adult male; Tshangkha, Tangsibji, Trongsa; 27.440°N, 90.420°E; 2161 m asl; 6 Jul. 2022; C. Dorji, Karma Chorten & Jigme leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5505; NBCB142.

Paratype. BHUTAN • 1 adult female; Namkye, Nabji, Korphoog, Trongsa; 27.166°N, 90.562°E; 2252 m asl; 11 Aug. 2022; Sanjit Rai leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5544; NBCB151.

Other material. BHUTAN • 2 nymph males; Tshangkha, Tangsibji, Trongsa; 27.440°N, 90.420°E; 2161 m asl; 6 Jul. 2022; C. Dorji, Karma Chorten & Jigme leg.; Cool broadleaf forest: under fallen tree branches and leaf litter.; MPN_CW5506, MPN_CW5507. BHUTAN • 1 female; Namkye, Nabji, Korphoog, Trongsa; 27.166°N, 90.562°E; 2252 m asl; 11 Aug. 2022; Sanjit Rai leg.; Cool broadleaf forest: under fallen tree branches and leaf litter; MPN_CW5545.

Description. Male. Body small in size (<16 mm). Median ocellus oval and lateral ocelli slightly oblong or oval, occupying basal two-thirds of lateral surface of the rostral tubercles. Posterior median margin of 9th abdominal tergite less rounded, embedded within 8th abdominal tergite (Fig. 16F). Tenth abdominal tergite with obtuse projection at the posterolateral margin with more convex shaped projection in the middle (Fig. 16F). Epiproct broad, less strongly curved but with two distinct grooves on the dorsal surface (Fig. 16F & G), with a pair of distal projections on the posterior median curved slightly upwards (Fig. 16E & G). Ventral basal plate of epiproct quadrate, with a pair of large lobes directed upward away from each other (usually backward when inserted to genitalia cavity) (Fig. 16E). These lobes are as long as the length of ventral basal plate of epiproct, with a pair of short sclerotised apical margin spines (Fig. 16E & H). Cerci slender and cylindrical, apices obtuse. Subgenital plate much wider than long forming oval lobes with wide space in between. Styli short, conical, arcuate with sclerotised acute apices (Fig. 16H). Linear measures of body elements (Table 4). Fore and mid leg spine details as per the generic description except mid tibiae with three superior linear spines (one prolateral and two retrolateral). Hind leg spine details (Table 5).

Colouration. Head and pronotum mostly dark brown or reddish and black dorsally. Body brown with numerous small pale brown or yellowish spots along posterior edges of thoracic and abdominal tergites (Fig. 16E), and slightly yellowish brown from beneath. Rostral tubercles dark brown. Cerci blackish in the centre and white on the ends.

Female. Larger than male (Table 4), body medium in size (>25 mm). Median and lateral ocelli same as male holotype. Subgenital plate much wider than long, simple, more convex than triangular, basal with small depression in the centre (Fig. 16J). Ovipositor base not so broad, long and gradually narrowing towards apex, curved, apices acute. Lower valves serrated on

distal area of ventral margin, and upper valves smooth. The general colouration is similar to male except body with more small brown/yellowish spots along posterior edges of thoracic and abdominal tergites. Linear measures of body elements (Table 4). Fore and mid leg spine details as per the generic description except mid tibiae with only three inferior linear spines (retrolateral). Hind leg spine details (Table 5).

Etymology. “*Bilobata*” from the Latin for ‘two lobes’ –referring to the specialised ventral basal plate of epiproct, which forms a pair of long lobes.

***Rhaphidophora piscicauda* Dorji, Morgan-Richards & Trewick sp. nov.**

(Figure 17A–H)

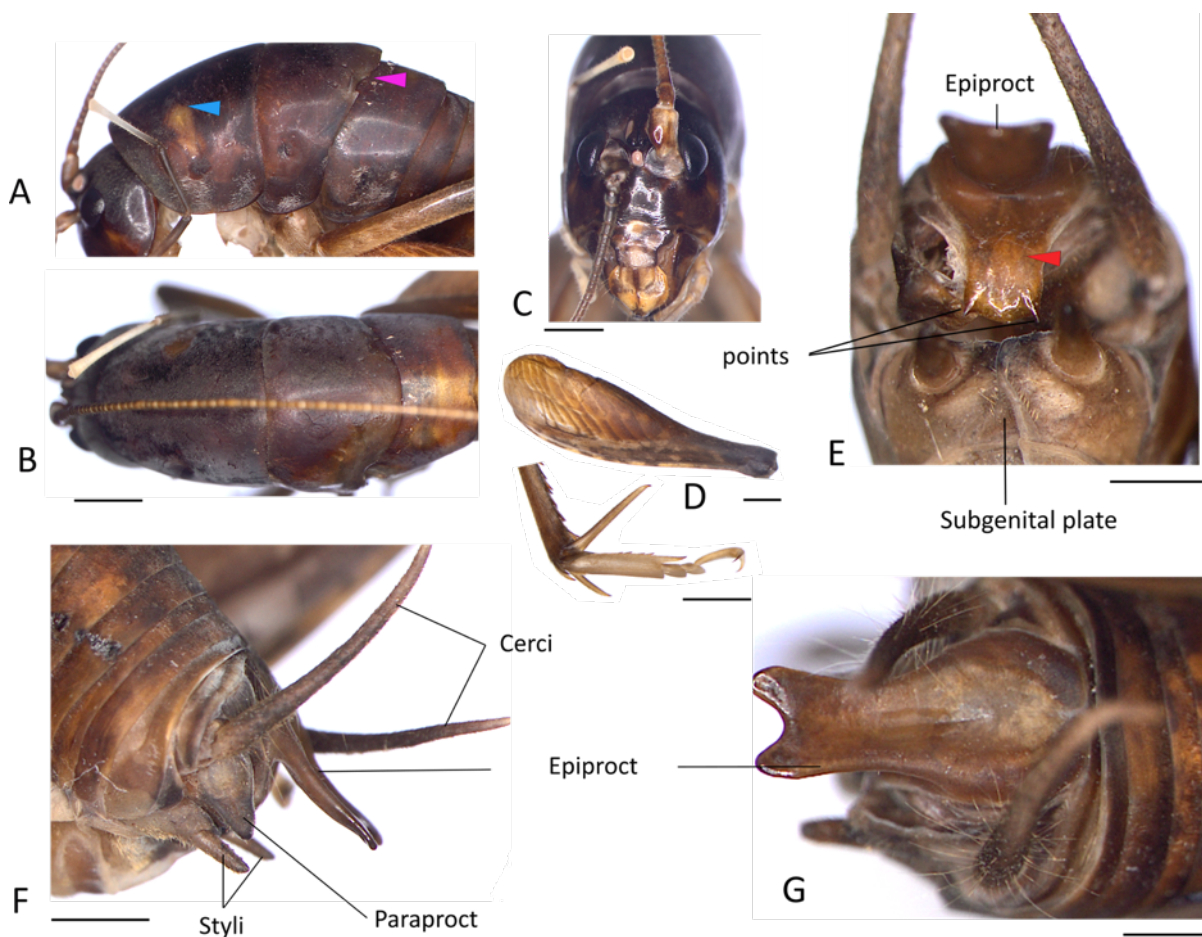


Figure 17. *Rhaphidophora piscicauda* sp. nov. Male holotype (MPN_CW5475; NBCB143): (A) Lateral and (B) dorsal views of body. (C) Anterior view of head. (D) Lateral view of hind femur and basitarsus. (E) Ventral view of lower abdomen showing quadrate shaped ventral basal plate of epiproct (red arrow) with a pair of sclerotised apical spines. (F) Lateral and (G) dorsal views of lower abdomen. Scale bars = 2 mm.

Holotype. BHUTAN • 1 adult male; Mahadev Dham, Tashiding, Dagana; 26.899°N, 89.975°E; 915 m asl; 21 May 2022; Jigme Wangchuk & Galey leg.; Subtropical forest: surrounding limestone cave under leaf litter; MPN_CW5475; NBCB143.

Other material. BHUTAN • 2 male and 1 female nymphs; Mahadev Dham, Tashiding, Dagana; 26.899°N, 89.975°E; 915 m asl; 21 May 2022; Jigme Wangchuk & Galey leg.; Subtropical forest: surrounding limestone cave; MPN_CW5474, CW5476, CW5477.

Description. Male. Body medium in size (<20 mm). Median ocellus elliptic or oblong (Fig. 17C) slightly longer than its width; lateral ocelli oval, occupying basal two-thirds of lateral surface of the rostral tubercles. Posterolateral margins on left side of mesonotum and metanotum are different from the right possibly because of incomplete ecdysis during nymph stages or early-stage injuries (Fig. 17A & B). Epiproct broad and long, fishtail shaped when view dorsally. The dorsal surface of epiproct is shallowly grooved (Fig. 17G), slightly curved laterally, projecting behind body. In about proximal half gradually narrowing with apical margin obtusely excised from the centre forming two lateral obtuse apices (Fig. 17G). Ventral surface of basal plate of epiproct is almost quadrate with slightly angled from middle on lateral view with a pair of small sclerotised marginal spines slightly projecting outwards, separated by traverse ridge (Fig. 17E). Cerci slender, conical and slightly longer than epiproct. Paraproct acutely triangular (Fig. 17F). Subgenital plate width as wide as length, with two distinct large lobes (Fig. 17E). Styli stout and simple, conical and apical obtuse without sclerotization. See table 4 for length of body elements and table 5 for spine details on legs. Linear measures of body elements (Table 4). Fore and mid leg spine details as per the generic description except mid tibiae with only three inferior linear spines (retrolateral). Hind leg spine details (Table 5).

Colouration. Head dorsally back with brownish face and mouthparts. Rostral tubercles dark black. Eyes silver white and black forming maculation of white and black connecting spots. Body dark brownish from above and slightly yellowish brown from beneath when compared to other species. Epiproct brownish. Cerci dark brown with one-third end white. Cerci and styli white with dense yellowish brown short setae, and fewer longer hairs along first half of the cerci.

Female. Adult unknown.

Etymology. Name is derived from fishtail shape of male epiproct in dorsal view.

Key to the Known Rhaphidophoridae of Bhutan

Superior apical spines of hind tibiae shorter than 1st and 2nd segments of hind tarsi combined. Male subgenital plate without styli..... Subfamily **Aemodogryllinae** Jacobson, 1905

Superior apical spines of hind tibiae longer than 1st and 2nd segments of hind tarsi combined. Male subgenital plate with styli..... Subfamily **Rhaphidophorinae** Walker, 1869

Subfamily Aemodogryllinae: Key to the known male Bhutanese *Diestramima*

- 1) Male posteromedian projection of 7th abdominal tergite long covering paraproct from above (Fig. 6E & 8E) **2**
 - Male posteromedian projection of 7th abdominal tergite short covering only proximal half of paraproct (Fig. 7E, 9E, & 12E)..... **4**
- 2) Male posteromedian projection of 7th abdominal tergite without prominent formation of lateral ridges (concave) in proximal half surface, apex obtusely rounded with inconspicuous notch; paraproct triangular with a short finger-like projection (Fig. 8C–E).....
 - *D. minjiwoonga* **sp. nov.**
 - Male posteromedian projection of 7th abdominal tergite rather high with lateral ridges in proximal half, apex distinctly notched (Fig. 6E, Fig. 10E, & Fig. 11A); paraproct long and finger-like projection in distal half (Fig. 6C, Fig. 10C, & Fig. 11C)..... **3**
- 3) Male posteromedian projection of 7th abdominal tergite gradually narrowing with distinct expanded notched apex; paraproct long with finger-like projection in distal half (Fig. 6C–E)
 - *D. tsongkhapa* (Würmli, 1973)
 - Male posteromedian projection of 7th abdominal tergite long, obviously surpassing paraproct, parallel from both sides, almost flat and curved downwards in distal half with apex curved inwards and acutely notched (Figs. 10C–F & Fig. 11B–C)
 - *D. phubdorjia* **sp. nov.**
- 4) Posteromedian projection of 7th abdominal tergite straight and flat but shallowly grooved from dorsomedial until apex, almost parallel lateral edges and truncate with small notch at median part of apex (Fig. 12B–C). *D. samkhara* **sp. nov.**
 - Male posteromedian projection of 7th abdominal tergite high and slightly curved gradually narrowing towards apex (Fig. 7E & Fig. 9E) **5**
- 5) Posteromedian projection of 7th abdominal tergite very short not extending beyond tenth tergite, gradually narrowing, truncate or slightly convex apex (Fig. 7B–D). Adult female larger than adult male..... *D. matermagna* **sp. nov.**
 - Male posteromedian projection of 7th abdominal tergite short covering proximal half of paraproct, narrowing to form almost parallel edges towards apex, truncate apex with slightly bent downwards (Fig. 9B–E). Superior prolateral and retrolateral edges of hind tibiae bear >50 minute spines *D. multidenticuli* **sp. nov.**

Subfamily Rhaphidophorinae: Key to the Known Male Bhutanese *Rhaphidophora*

- 1) Male epiproct more or less specialised bearing a projection or a curved, narrowed or bifurcate apex (Fig. 14C, Fig. 16H, & Fig. 17G).....2
- Male epiproct simple without long projection, disc oval, carinated all around, only at apex almost flat surface with a pair of short lateral spines directing downwards (Fig. 15F–G); ventral basal plate of epiproct triangular, gradually forming into single sclerotised spine (Fig. 15E) *R. bicuspis* **sp. nov.**
- 2) Male epiproct less specialised, longer than wide; ventral basal plate of epiproct without any evident projections or specialisation3
- Male epiproct short slightly curved upward at apex (Fig. 14E & Fig. 16F), ventral basal plate of epiproct specialised (Fig. 14F & Fig. 16H)4
- 3) Male epiproct acute-triangular, disc carinated all around with a longitudinal groove towards apex, apex projecting behind elevated disc and with 2 obtuse dorsal small lobes
.....*R. angulata* Ingrisch, 2002
- Male epiproct longer than wide, dorsal surface shallowly curved projecting behind body; basal half broad and almost parallel thereafter with 2 obtuse lateral apices (Fig. 17F–G).
.....*R. piscicauda* **sp. nov.**
- 4) Male epiproct oval, lateral margins not strongly curved but grooved dorsally with a conspicuous pair of projections on distal end directed slightly backwards; ventral basal plate of epiproct with a pair of swollen lobes directed upward and a small triangular sclerotised apical lobes (Fig. 14E–G).....*R. bhutanensis* **sp. nov.**
- Male epiproct oval, lateral margins less curved but deeply grooved in dorsal view and with a pair of projections on the distal end directed slightly upwards; ventral basal plate of epiproct with a pair of large lobes directed upward away from each other, apical with a pair of sclerotised ventral margin spines (Fig. 16E–H).....*R. bilobata* **sp. nov.**

DISCUSSION**Systematics**

More than half of the global raphidophorid diversity is within Asian lineages with about 800 different species in 38 genera (Cigliano et al., 2023). Only two species were reported from Bhutan; *Diastramima tsongkhapa* and *Rhaphidophora angulata* (Ingrisch, 2002; Würmli, 1973). Here we describe nine new species taking the total raphidophorid diversity to 11 species native to the eastern Himalaya of Bhutan. This was possible by searching a wider range of habitats and a larger area within Bhutan than ever before. The true number of species in the country is undoubtedly much higher, as vast areas of Bhutan's territory remain insufficiently studied.

Our phylogenetic inference based on mtDNA aligns with morphological findings, demonstrating that species within the two subfamilies Rhabdophorinae and Aemodogryllinae form distinct clades. The genus *Diestramima* is distinguished from other genera within the tribe Diestramimini by the presence of a long male posteromedial projection on the 7th abdominal tergite, which typically extends over the paraprocts from above, and by membranous male genitalia featuring eight lobes (Gorochov & Storozhenko, 2015). Based on the male 7th abdominal tergite, 12 of 45 specimens closely aligned with this description: *D. phubdorjia* **sp. nov.** (n = 4), and *D. tsongkhapa* (n = 8). The remaining specimens had a relatively short 7th abdominal tergite, extending only to the proximal half of the paraprocts without completely covering them. Despite this variation from what is regarded as a diagnostic trait for the genus our mtDNA sequences support all specimens as part of the same genus. Morphological variation in the shape of abdominal tergites concordant with the genetic clusters resolved five new species in the genus *Diestramima*: *D. matermagna* **sp. nov.**, *D. minjiwoonga* **sp. nov.**, *D. multidenticuli* **sp. nov.**, *D. phubdorjia* **sp. nov.**, and *D. samkhara* **sp. nov.**

The genus *Diestramima* has been further subdivided into three subgenera based on the distinct shapes of paraprocts (Gorochov & Storozhenko, 2019). The distal half of male paraproct of subgenus *Baculitettix* is curved, long and finger-like, with the proximal half triangular. In subgenus *Diestramima* the male paraproct is rather short and plate-like with distal part truncate, angular or having hooks and/or teeth, whereas the male paraproct of subgenus *Excisotettix* is moderately short, almost S-shape in profile, with proximal and distal parts more or less equal to each other in height, and with angular or almost spinose apex. However, all the species described from Bhutan possess a simple triangular paraproct without distinct structural morphology that can be associated to either of the subgenera (Fig. 18). *D. matermagna* **sp. nov.**, *D. minjiwoonga* **sp. nov.**, and *D. multidenticuli* **sp. nov.** have paraprocts somewhat similar to that of subgenus *Baculitettix* but they are more acutely triangular without any finger-like distal part. Instead, it gradually and evenly narrows towards the apex with slight curvature at the dorsal mid region. *D. samkhara* **sp. nov.** male paraprocts are comparatively stout, and more triangular with slight dorsal curvature towards apex. *D. phubdorjia* **sp. nov.** is the only species which can be grouped into subgenus *Baculitettix* that has a finger-like distal half of paraproct but not long as much as in *D. tsongkhapa*. Due to the variation in paraproct morphology in *Diestramima* species in Bhutan, these species could not be assigned to existing subgenera, nor was a new subgenus proposed. Molecular phylogenetics does not resolve the subgenera classification providing another reason to be cautious of this taxonomic level (Fig.5).

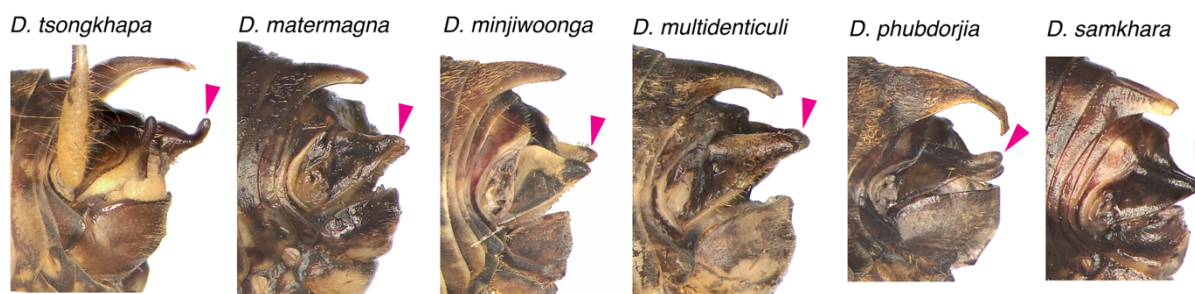


Figure 18. Lateral view of terminalia of males of *Diestramima* species from Bhutan showing differences in paraproct shape (pink arrows).

Genus classification within Rhaphidophorinae is based on the presence or absence of a projection on the abdominal tergites in male. Among these, *Rhaphidophora*, characterized by the absence of abdominal projection and a simple epiproct is the most speciose. Specimens from Bhutan, represented by mtDNA sequences were resolved into clusters concordant with morphology and confirming association of females, nymphs and adult males. Thus, four new species are described within the genus *Rhaphidophora* based on the distinct shape of the epiproct and our genetic evidence. The epiproct of male *R. bicuspis* **sp. nov.** is the simplest in shape of the four species, with two distinct marginal spines at its apex. Four specimens were collected from Mahadev Dham (Dagana), and these form a distinct genetic cluster (Fig. 13). Three specimens were nymphs, but the single adult male allowed us to described *R. piscicauda* **sp. nov.** based on its distinct large, elongated, fishtail epiproct (Fig. 17). *R. piscicauda* **sp. nov.** resembles the Nepalese *R. stridulans* but differs in the shape of styli and epiproct (Ingrisch, 2006). *R. stridulans* styli are more curved and sclerotized with compress and acute apices. The proximal half of epiproct is more triangular than oval with the dorsal surface curved rather than almost flat or shallowly grooved in the case of new species. Moreover, the ventral basal plate of epiproct of the *R. piscicauda* **sp. nov.** is quadrate with the surface not concaved but instead flat or slightly angled at the midpoint in lateral view. The epiprocts of *R. bilobata* **sp. nov.** and *R. bhutanensis* **sp. nov.**, somewhat resemble that of genus *Neorhaphidophora*. The epiproct is short and grooved dorsally with apices slightly curved upward, whereas the ventral plate of epiproct is specialised to form a pair of lobes which is quite distinct in *R. bilobata* **sp. nov.** (Fig. 15 & Fig. 17). The genus *Neorhaphidophora* was established for species characterized by a strongly modified male epiproct featuring a pair of large basal projections directed backwards and slightly upwards, with bent downward and forward so that it remains inserted in the genital cavity (Gorochoy, 2011). Currently, *Neorhaphidophora* is known from countries neighbouring Bhutan such as Myanmar, China and Thailand suggesting a high likelihood that it is also present

in Bhutan. Currently there are no genetic data available that would allow comparison of our specimens to species placed within *Neorhaphidophora*, so despite minor modification of epiproct, *R. bilobata* **sp. nov.** and *R. bhutanensis* **sp. nov.** are here placed into *Rhaphidophora*. Furthermore, all the *Rhaphidophora* species described in this study fall within Group 1 (Gorochoy, 2012), characterized by the presence of only two apical spines on fore tibia and more or less specialised male epiproct with a projection or a curved, narrowed or bifurcate apical area.

Distribution and Habitat

Rhaphidophorids in arid and semi-arid habitats are often associated with caves, though many species are known to inhabit open forests, seldom using caves for refuge. Asian lineages of Aemodogryllinae, Rhaphidophorinae, and Anoplophilinae primarily dwell in temperate and tropical forests, with the exception of certain *Tachycines* and *Diestrammena* species, which are obligate cave dwellers across East Asia. To sample cave-adapted rhaphidophorids we explored three cave systems in Bhutan with well-developed dark zones: Rangtsi (southwest), Meritsemu (south), and Dugphu (central). However, we found few rhaphidophorids in caves of Bhutan but instead collected individuals among rocks at cave entrances. In Bhutan camel crickets were most commonly found in forest leaf litter, beneath fallen branches and rocks, within cliff crevices, and near cave entrances.

We conducted our survey across more than 50 sites in 17 districts, but despite extensive search effort, we encountered rhaphidophorids at only 15 sites across 10 districts (Fig. 4) in central and eastern regions. It is likely that our failure to find rhaphidophorids in other sites is largely due to searching during daylight. Notably, a recent night survey of a previously explored site at Rangtsi Nye (Haa), resulted in detection of many individuals of *Diestramima* species. Similarly, *D. tsongkhapa*, described from Khotokha (Wangdue Phodrang) was also resampled from Nabji (Trongsa) and Wangphu (Samdrup Jongkhar). This suggests that their distribution may be broader than our initial surveys indicated, emphasizing the need for further nocturnal investigations. However, due to inaccessibility of motor road and the remoteness of the area, night search often poses risks from wild animals and the dangers of monsoon torrential rain.

The study of Bhutanese rhaphidophorids remains in its early stage, and a comprehensive understanding of their diversity and geographical distribution is yet to be established. Rhaphidophorids of Bhutan represent the western extent of the group in the eastern Himalaya. The findings from our recent survey indicate that *D. tsongkhapa* is widely distributed across

central and eastern Bhutan, where it occurs sympatrically with *R. bilobata* **sp. nov.** (Trongsa). *D. matermagna* **sp. nov.** was found in sympatry with *R. bhutanensis* **sp. nov.** at Marung (Pema Gatshel) and appears to be more abundant in parts of the eastern region, with its presence recorded across three adjacent districts (Trashigang, Samdrup Jongkhar, and Pema Gatshel). The remaining species, particularly those of the genus *Rhaphidophora*, have been found in only a single locality which may indicate these are restricted to much smaller geographical regions.

Among the species described, *R. bicuspis* **sp. nov.** was found to be in the northernmost region of Bhutan, on south-facing slope of Lamperi (Thimphu) at an elevation of 2600 metres. Lamperi lies just below Dochula Pass (3,300 m asl), sub-alpine zone characterized by extreme cold and prolonged snow cover during the winter months. Rhaphidophorids are known for their ability to withstand harsh environmental conditions, including extreme heat and prolonged freezing temperatures (Hegg et al., 2022). The presence of *R. bicuspis* **sp. nov.** in such an environment suggests that the species may either possess physiological adaptations that allow it to survive freezing temperatures or might synchronise its life cycle to avoid seasonal extremes in temperatures. This discovery indicates the potential existence of cold tolerant raphidophorid species in the Himalayan mountains, a region where alpine biodiversity remains largely unexplored. Future research and exploration in these high-elevation ecosystems may lead to significant discoveries, contributing to a deeper understanding of insect adaptation and survival strategies in extreme environments.

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<https://doi.org/10.11646/zootaxa.4981.3.9>

SUPPLEMENTARY INFORMATION

Table S1. Number of fore and mid leg spines of type specimens from Bhutan (genus *Diestramima*).

Leg element	Spines	<i>D. matermagna</i> sp. nov.		<i>D. minjiwoonga</i> sp. nov.		<i>D. multidenticuli</i> sp. nov.		<i>D. phubdorjia</i> sp. nov.		<i>D. samkhara</i> sp. nov.		<i>D. tsongkhapa</i>	
		1 male	1 female	1 male		1 male	1 female	1 male	1 female	1 male	1 female	1 male	1 female
Fore femur	Apical (retro)	1	1	1		1	1	1	1	1	1	1	1
Fore tibia	Inferior linear (pro, retro)	2,2	2,2	2,2		2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2
	Superior apical (retro)	1	1	1		1	1	1	1	1	1	1	1
	Inferior apical (pro, ventral, retro)	1,1,1	1,1,1	1,1,1		1,1,1	1,1,1	1,1,1	1,1,1	1,1,1	1,1,1	1,1,1	1,1,1
Mid femur	Apical (pro, retro)	1,1	1,1	1,1		1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1
Mid tibia	Inferior linear (pro, retro)	2,2	2,2	2,2		2,2	2,2	2,2	2,2	2,2	2,2	2,2	2,2
	Superior subapical (pro, retro)	1,1	1,1	1,1		1,1	1,1	1,1	1,1	1,1	1,1	1,1	1,1
	Apical inferior (pro, central, retro)	1,1,1	1,1,1	1,1,1		1,1,1	1,1,1	1,1,1	1,1,1	1,1,1	1,1,1	1,1,1	1,1,1

Table S2. Number of fore and mid leg spines of type specimens from Bhutan (Genus *Rhaphidophora*).

Leg element	Spines	<i>R. bhutanensis</i> sp. nov.	<i>R. bicuspis</i> sp. nov.	<i>R. bilobata</i> sp. nov.		<i>R. piscicauda</i> sp. nov.
		1 male	1 male	1 male	1 female	1 male
Fore femur	Inferior apical (pro)	1	1	1	1	1
Fore tibia	Inferior linear (pro, retro)	1,2	1,2	1,2	1,2	1,2
	Apical (pro, retro)	1,1	1,1	1,1	1,1	1,1
Mid femur	Inferior apical (pro, retro)	1,1	1,1	1,1	1,1	1,1
Mid tibia	Superior linear (pro, retro)	2,2	1,1	1,2	2,2	2,2
	Inferior linear (pro, retro)	3,2	3,2	3,2	3,0	3,0
	Inferior subapical (pro, retro)	1,1	1,1	1,1	1,1	1,1
	Apical superior (pro, retro)	1,1	1,1	1,1	1,1	1,1

Table S3. Summary of genetic data submitted to GenBank with accession numbers.

Subfamily	Species	Locality	Voucher Code	GenBank Ac. no.
Aemodogryllinae	<i>Diestramima matern magna</i> sp. nov.	Marung Bridge, Pema Gatshel	MPN_CW5479	PV593689
			MPN_CW5490	PV593700
			MPN_CW5491	PV593698
			MPN_CW5536	PV593687
			MPN_CW5537	PV593688
			MPN_CW5539	PV593690
		Wangphu, Samdrup Jongkhar	MPN_CW5486	PV593699
			MPN_CW5488	PV593697
		Yurung Bridge, Pema Gatshel Kharungla Trashigang	MPN_CW5489	PV593696
			MPN_CW5495	PV593692
			MPN_CW5496	PV593693
			MPN_CW5497	PV593695
			MPN_CW5498	PV593694
			MPN_CW5499	PV593691
			MPN_CW5484	PV593667
	<i>Diestramima minjiwoonga</i> sp. nov. <i>Diestramima phubdorjia</i> sp. nov.	Minjiwoong Peg, Samdrup Jongkhar	MPN_CW5523	PV593669
		Nabji, Trongsa	MPN_CW5508	PV593670
		Tshangkha, Trongsa	MPN_CW5509	PV593671
	<i>Diestramima multidenticul</i> sp. nov.	Bawoong, Trashi Yangtse	MPN_CW5510	PV593668
			MPN_CW5533	PV593681
			MPN_CW5534	PV593682
	<i>Diestramima samkhara</i> sp. nov.	Samkhar Bridge, Sarpang	MPN_CW5535	PV593680
			MPN_CW5478	PV593683
			MPN_CW5541	PV593684
	<i>Diestramima tsongkhapa</i>	Philligang, Nabji, Trongsa	MPN_CW5542	PV593685
			MPN_CW5543	PV593686
			MPN_CW5516	PV593675
		Philligangju, Nabji, Trongsa	MPN_CW5517	PV593676
			MPN_CW5518	PV593672
			MPN_CW5520	PV593677
			MPN_CW5521	PV593679
			MPN_CW5522	PV593678
			MPN_CW5524	PV593673
			MPN_CW5528	PV593674
			MPN_CW5483	PV593660
	<i>Rhaphidophora bhutanensis</i> sp. nov.	Namkey, Nabji, Trongsa	MPN_CW5470	PV593661
		Marung Bridge, Pema Gatshel	MPN_CW5529	PV593662
		Bali-Pothey, Tsirang		
		Lamperi, Thimphu		
	<i>Rhaphidophora bicuspis</i> sp. nov. <i>Rhaphidophora bilobata</i> sp. nov.	Nabji, Trongsa Tshangkha, Trongsa	MPN_CW5545	PV593659
			MPN_CW5505	PV593658
			MPN_CW5506	PV593656
	<i>Rhaphidophora piscicauda</i> sp. nov.	Mahadev Dham, Dagana	MPN_CW5507	PV593657
			MPN_CW5474	PV593666
			MPN_CW5475	PV593664
			MPN_CW5476	PV593665
Rhaphidophorinae			MPN_CW5477	PV593663

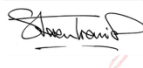
Table S4. Details of Rhaphidophoridae specimens collected in Bhutan in 2022.

Locality Name	Gewog	District	Latitude	Longitude	Elevation (m)	Date	leg	Habitat description	Unique code
Bali-Pothey	Kilkhorthang	Ts	27.464°N	90.059°E	1266	1.v.2022	C. Dorji & Yeshe Phuntsho	Temperate forest: Under two huge rock boulders.	MPN_CW5469 MPN_CW5470 MPN_CW5471 MPN_CW5472 MPN_CW5473 MPN_CW5474 MPN_CW5475
Mahadev Dham	Tashiding	Da	26.899°N	89.975°E	915	21.v.2022	Jigme Wangchuk & Galey	Subtropical forest: surrounding limestone cave.	MPN_CW5476 MPN_CW5477
Mahadev Dham	Tashiding	Da	26.899°N	89.975°E	916	21.v.2023	Jigme Wangchuk & Galey	Subtropical forest: surrounding limestone cave.	MPN_CW5478
Samkhara Bridge	Gelephu	Sp	27.009°N	90.581°E	942	22.v.2022	C. Dorji & Jigme	Subtropical forest: Under boulders above bridge.	MPN_CW5480 MPN_CW5481
Marung Bridge	Khar	PG	27.011°N	91.420°E	923	25.v.2022	C. Dorji, Tshewang Namgay & Jigme	Subtropical forest: cliff base under leaf litter and small rocks.	MPN_CW5482
Marung Bridge	Khar	PG	27.011°N	91.420°E	924	25.v.2023	C. Dorji, Tshewang Namgay & Jigme	Subtropical forest: cliff base under leaf litter and small rocks.	MPN_CW5483
Minjiwoong Peg	Serthi	SJ	26.988°N	92.012°E	833	28.v.2022	C. Dorji, Tshering Dorji & Jigme	Subtropical forest: cave entrance.	MPN_CW5484
Wangphu	Gomdar	SJ	26.982°N	91.584°E	1028	29.v.2022	C. Dorji & Jigme	Subtropical forest: limestone cave entrance	MPN_CW5485 MPN_CW5486 MPN_CW5487 MPN_CW5488 MPN_CW5489
Yurung Bridge	Khar	PG	27.040°N	91.380°E	571	31.v.2022	C. Dorji, Tshewang Namgay & Jigme	Subtropical forest: cliff base under leaf litter and small rocks.	MPN_CW5490 MPN_CW5491
Marungri	Khar	PG	27.011°N	91.420°E	923	31.v.2022	C. Dorji & Jigme	Subtropical forest: cliff base under crevices and leaf litter.	MPN_CW5492 MPN_CW5493 MPN_CW5494 MPN_CW5495 MPN_CW5496 MPN_CW5497 MPN_CW5498 MPN_CW5499 MPN_CW5500 MPN_CW5501 MPN_CW5502 MPN_CW5503 MPN_CW5504
Kharungla	Brekha	TG	27.155°N	91.628°E	2039	1.vi.2022	C. Dorji & Jigme	Cool broad leaf forest: surrounding huge boulders and nearby vegetation.	MPN_CW5505 MPN_CW5506 MPN_CW5507 MPN_CW5508 MPN_CW5509 MPN_CW5510 MPN_CW5511 MPN_CW5512 MPN_CW5513
Bawoong	Ramjar	TY	27.416°N	91.586°E	1582	2.vi.2022	C. Dorji & Jigme	Temperate forest: under boulders and surrounding vegetation.	MPN_CW5515 MPN_CW5516 MPN_CW5517 MPN_CW5518 MPN_CW5519 MPN_CW5520 MPN_CW5521 MPN_CW5522 MPN_CW5523 MPN_CW5524
Tshangkha	Tangsibji	Tr	27.440°N	90.420°E	2161	6.vi.2022	C. Dorji, Karma Chorten & Jigme	Cool broadleaf forest: under fallen tree branches and leaf litter.	MPN_CW5525 MPN_CW5526 MPN_CW5527 MPN_CW5528
Nakha	Korphoog	Zh	27.117°N	90.799°E	1089	9.iv.2022	Tenzin Drukgyel	Cool broadleaf forest: under fallen tree branches and leaf litter.	MPN_CW5529
Nabji	Korphoog	Tr	27.178°N	90.535°E	1381	30.vi.2022	Sanjit Rai	Cool broadleaf forest: under fallen tree branches and leaf litter.	
Nabji	Korphoog	Tr	27.180°N	90.535°E	1305	30.vi.2022	Sanjit Rai	Cool broadleaf forest: under fallen tree branches and leaf litter.	
Nabji	Korphoog	Tr	27.165°N	90.562°E	2252	22.vi.2022	Sanjit Rai	Cool broadleaf forest: under fallen tree branches and leaf litter.	
Lamperi		Th	27.498°N	89.767°E	2761	22.vii.2022	C. Dorji	Cool broadleaf forest: forest floor under leaf litter.	

Table S4. Details of Rhabdophoridae specimens collected in Bhutan in 2022 (*cont.*).

Locality Name	Gewog	District	Latitude	Longitude	Elevation (m)	Date	leg	Habitat description	Unique code
Bawoong	Ramjar	TY	27.416°N	91.586°E	1582	2.vi.2022	C. Dorji & Jigme	Temperate forest: under boulders and surrounding vegetation.	MPN_CW5531 MPN_CW5532 MPN_CW5533 MPN_CW5534 MPN_CW5535
Marung Bridge	Khar	PG	27.009°N	91.421°E	936	31.v.2022	C. Dorji & Jigme	Subtropical forest: cliff base under leaf litter and small rocks.	MPN_CW5536 MPN_CW5537 MPN_CW5538 MPN_CW5539 MPN_CW5540
Samkhar Bridge	Gelephu	SP	27.009°N	90.581°E	942	22.v.2022	C. Dorji & Jigme	Subtropical forest: Under boulders above bridge.	MPN_CW5541 MPN_CW5542 MPN_CW5543
Nabji	Korphoog	Tr	27.166°N	90.562°E	2252	11.vii.2022	Sanjit Rai	Cool broadleaf forest: under fallen tree branches and leaf litter.	MPN_CW5544 MPN_CW5545

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CHAPTER THREE

Cretaceous connections among camel cricket lineages in the Himalaya revealed through fossil-calibrated mitogenomic phylogenetics

SIMPLE SUMMARY

The flightless camel crickets are one of the oldest living lineages of Orthoptera, believed to have originated around 250 million years ago. In this paper we infer the timing of their radiation using DNA sequences from whole mitochondrial genomes of 20 camel crickets, with a focus on the neglected Rhaphidophorinae and Aemodogryllinae groups. To determine whether the taxonomic groups share a single common ancestor, we combined new DNA sequences from camel crickets from Bhutan with published genetic data. Our phylogenetic tree supports the monophyly of most of the genera sampled but supports the reinstatement of *Gymnaeta* Adelung, which forms a lineage sister to the group comprising *Diestrammena*, *Tachycines*, *Gymnaetoides*, *Homotachycines*, and *Pseudotachycines*. Based on our fossil-calibrated molecular clock phylogeny, the common ancestor of camel crickets was estimated to have lived in the Early Jurassic when the supercontinents were still connected. We estimate that the most recent common ancestor of Aemodogryllinae and Rhaphidophorinae lived about 137 million years ago, well before America and Asia were connected by the Bering Land Bridge. Thus, we find little evidence to suggest that continental drift explains the current distribution of this wingless orthopteran family.

ABSTRACT

The nocturnal, flightless camel crickets (Rhaphidophoridae) have a global distribution and are believed to have originated prior to the breakup of Pangea. We investigated the phylogeny and the timing of the radiation of East Asian species with mitogenomic data. Initially we analyzed a large taxon dataset ($n = 117$) using available partial mitochondrial and nuclear DNA sequences to confirm the monophyly of subfamilies and current taxonomy. Our findings support the monophyly of each genus within the subfamily Aemodogryllinae, with a minor inconsistency between taxonomy and phylogeny resolved by resurrection of the genus *Gymnaeta* Adelung. Fossil-calibrated molecular clock analysis used 11,124 bp alignment of 13 complete mitochondrial protein-coding genes for 20 species of *Rhaphidophoridae*, with a focus on the neglected Rhaphidophorinae and Aemodogryllinae lineages. Divergence time estimates suggest that the most recent common ancestor of the family lived during the Early Jurassic ($189 \text{ Mya} \pm 23 \text{ Mya}$) before Pangea broke into the supercontinents or possibly during the early stage of breakup when Gondwana and Laurasia were still connected by land. The two subfamilies, Rhaphidophorinae and Aemodogryllinae, that overlap in Asia are estimated to have diverged $138 \text{ Mya} \pm 17 \text{ Mya}$, well before the Late Cretaceous northern connection between America and Asia (the Bering Land Bridge). Thus, our extended sampling of species from East Asia and Oceania refutes the importance of continental drift in the evolution of this wingless orthopteran family.

KEYWORDS

Rhaphidophoridae; molecular clock; phylogeny; camel cricket; cave cricket; sympatry.

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INTRODUCTION

The Rhaphidophoridae, commonly known by the names camel crickets, cave crickets, and cave wētā, are among the least-studied groups within the Orthoptera (Song et al., 2015). The family is diverse, encompassing 912 extant described species and 93 genera (Cigliano et al., 2023) distributed around the world. Although all species of Rhaphidophoridae are flightless, there are numerous examples living on geologically young oceanic islands (Trewick, 2000; Allegrucci & Sbordoni, 2019) resulting from colonization, which demonstrates their excellent dispersal potential (Dowle et al., 2024). They are characterized by an arched profile; long antennae; and usually long legs, an absence of wings and tympanum, and limited pigmentation (Figure 1). They are nocturnal and associated with humid environments in temperate and tropical wet forests and sometimes in caves.

The Rhaphidophoridae are classified into nine extant and one extinct subfamily, each restricted to a distinct geographic region (Figure 1): the Troglophilinae and Dolichopodainae are found in Mediterranean Europe; the Rhaphidophorinae, Aemodogryllinae, and Anoplophilinae in Asia, with the latter being limited to the Korean Peninsula and Japan; the Macropathinae in the Southern Hemisphere; and the Ceuthophilinae, Gammarotettiginae, and Tropidischinae exclusively in North America. Although representation in molecular phylogenetic analyses is sometimes sparse, the monophyly of seven subfamilies is concordant with their distinct geographical distributions (Allegrucci & Sbordoni, 2019; Kim et al., 2024). The geographic partitioning of relatively deep lineages implies long regional persistence in separate regions, but this pattern does not necessarily reveal paleogeographic processes due to the unknown influence of extinction (Grandcolas & Trewick, 2016).

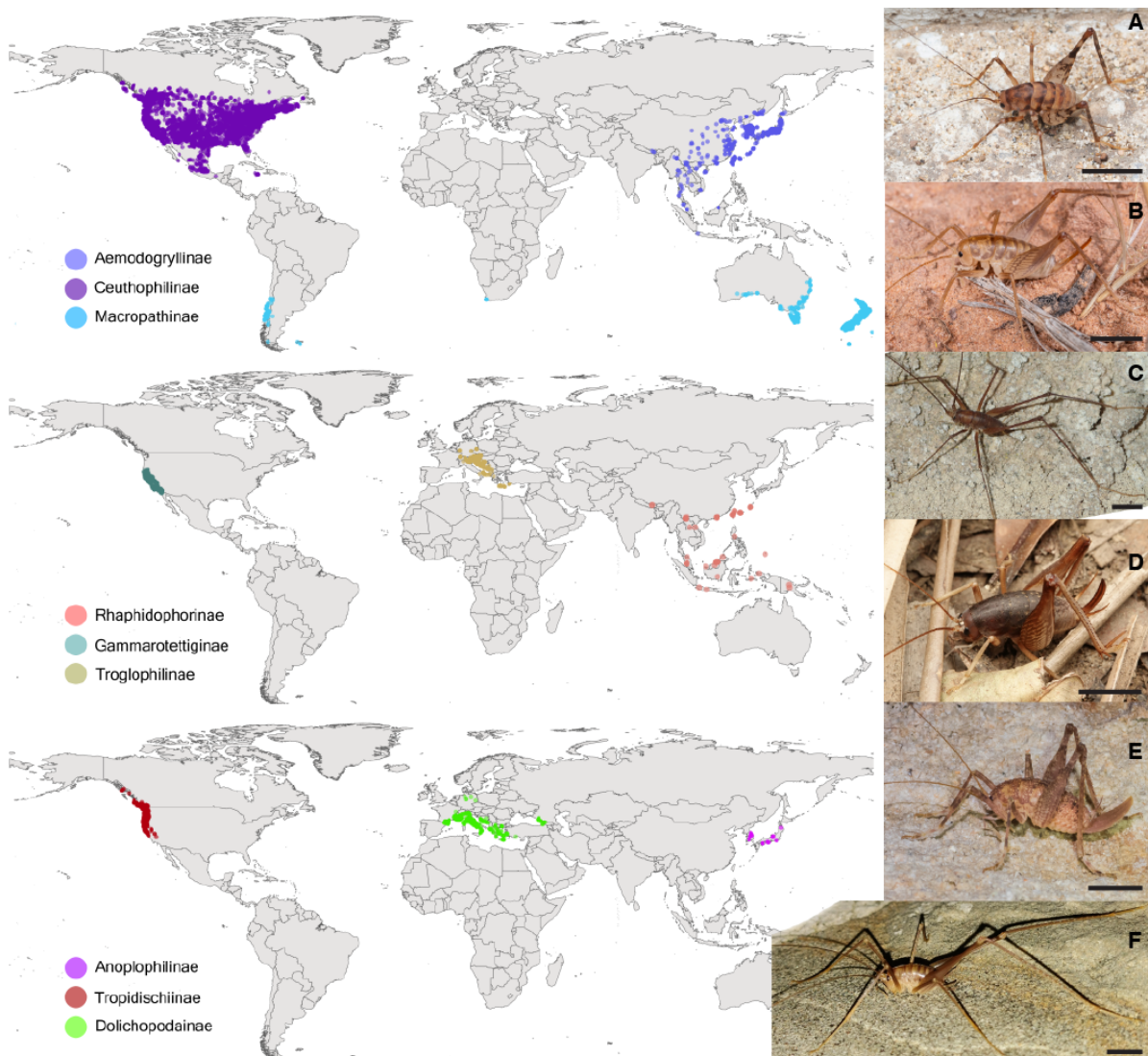


Figure 1. Global distribution of raphidophorid subfamilies based on 23,862 locality records retrieved from iNaturalist, GBIF (<https://www.gbif.org/>) and BOLD System (<https://v3.boldsystems.org/>). Data processed in QGIS v3.22 and edited in Inkscape v1.2.1. The subfamily Tropidischinae comprises a single species (*Tropidischia xanthostoma*) of uncertain affinity. The invasive range of the greenhouse raphidophorid *Tachycines asynamorus* in North America and Europe is excluded. (A) Aemodogryllinae: male *Tachycines asynamorus*, China (Zhangyiyan). (B) Ceuthophilinae: female *Ceuthophilus* sp., USA (Danilo Hegg). (C) Dolichopodainae: female *Dolichopoda* sp., Italy (Fabrizio Mujica). (D) Rhaphidophorinae: female *Rhaphidophora taiwana* Okinawa, Japan (Orthoptera-JP). (E) Troglophilinae: female *Troglophilus cavicola*, Italy (Marco Vicariotto). (F) Macropathinae: male *Macropathus filifer*, NZ.

The Raphidophoridae are regarded as a sister to the majority of ensiferan diversity and are thought to have originated between the Carboniferous and Late Jurassic periods (Hubbell & Norton, 1978; Ragge, 1955; Sharov, 1971). Vicariant explanations of the global distribution of

the Raphidophoridae rather than long-distance dispersal have historically been favored, primarily due to the absence of wings in all species (Ander, 1939; Karny, 1934; Richards, 1965), and phylogenetic trees are broadly consistent with this (Allegrucci et al., 2010, 2011, 2017; Allegrucci & Sbordoni, 2019; Kim et al., 2024; Zhu, Zhou, et al., 2022). While Northern Hemisphere subfamilies are each associated with one part of a continent, the Macropathinae are distributed across multiple continents, including South Africa, South America, Australia, New Zealand, and several subantarctic islands. Data-rich phylogenetic analyses of this subfamily show that raphidophorids have successfully dispersed long-distances across inhospitable habitats (Dowle et al., 2024), and it is widely recognized that dispersal is the only plausible explanation for the current spatial arrangement of many endemic species on young oceanic islands (Bargelloni et al., 2000; Cowie & Holland, 2006; Trewick & Morgan-Richards, 2005; Waters & Craw, 2006). Sister to the Macropathinae are the Northern Hemisphere subfamilies the Aemodogryllinae and Raphidophorinae, which have their nexus in Southeast Asia, a geologically complex region of continent and islands. How did these distinctive lineages come to occupy the same region? One suggestion is that their common ancestor colonized East Asia in the later Cretaceous via the Bering Land Bridge (Kim et al., 2024).

The timing of lineage origins and the relevance of landscape processes in the evolution of the Orthoptera and other insects often relies on the application of paleogeographic events for phylogenetic dating (Allegrucci & Sbordoni, 2019; Borissov et al., 2023; Trichas et al., 2020), but this is problematic since it is founded on an assumption of the very question to be decided (Wallace, 1876); it assumes a role of landscape changes in lineage formation, which results in logical circularity (Crisp et al., 2011; Trewick, 2011; Waters & Craw, 2006). However, the scarcity of relevant verified fossils inhibits the use of this alternative to calibration (Ho & Phillips, 2009).

The earliest fossils assigned to the Raphidophoridae are from Oligocene Baltic amber (Chopard, 1936). *Protroglophilus* and *Prorhaphidophora* (Chopard, 1936; Gorochoy, 1989) represent lineages of uncertain affinity and are not definitively stem lineages of the family or extant subfamilies and are therefore unsuitable for molecular phylogeny that seeks to estimate the divergence of the major phylogenetic lineages. Uncertainty in the systematic placement of other extinct taxa in the Tettigoniidea, such as *Aenigmaraphidophora mouniri* (Azar et al., 2022), prevents their use. Consequently, previous molecular phylogenetic studies on the Raphidophoridae used inferred paleogeographic events to calibrate the most recent common

ancestor of the group (Allegrucci et al., 2010; Allegrucci & Sbordoni, 2019; Beasley-Hall et al., 2018). To overcome the limited availability of fossils, ‘secondary fossil’ calibration relies on fossil-constrained calibration of a different taxon set that overlaps the group of interest, with node dates being transferred for molecular clock analysis of the focal group (Kim et al., 2024; Zhu, Zhou, et al., 2022).

Camel crickets from the Asian continent form a major part of the global Rhaphidophoridae diversity, yet their phylogenetic relationships have been neglected. For *Tachycines* and *Diestrammena* within the subfamily Aemodogryllinae, classification of species is challenging due to the large number of species, their high degree of morphological similarity, and the lack of diagnostic characters. The genus *Tachycines* was established with *T. asynamorus* by separation from *Diestrammena* based on a difference in the number of spines on the hind tibia (Adelung, 1902). Furukawa, in 1933, did not accept the evidence for the division of *Diestrammena* and *Tachycines* proposed by Adelung and preferred *D. (Tachycines)* (Furukawa, 1933). Karny (1934) merged *Diestrammena* with *Gymnaeta* Adelung as subgenera of *Tachycines*; hence *Tachycines (Diestrammena)* and *T. (Gymnaeta)* (Karny, 1934). Gorochov and Storozhenko (1992) transferred the subgenus *Gymnaeta* from the genus *Tachycines* to *Diestrammena* (Gorochov & Storozhenko, 1992). Six years later, Gorochov (1998), using male genitalia, proposed four subgenera of *Diestrammena*: *D. (Diestrammena)*, *D. (Aemodogryllus)*, *D. (Tachycines)*, and *D. (Gymnaeta)* (Gorochov, 1998) (Qin, Wang, et al., 2018; Zhang & Liu, 2009). Despite the complicated and convoluted taxonomic history of these cricket species, their systematics has not been examined using molecular phylogenetics.

We sampled taxa representing five subfamilies to determine the evolutionary relationships of the Rhaphidophoridae, including systematic analysis of neglected Asian subfamilies, the Aemodogryllinae and Rhaphidophorinae. We constructed phylogenetic hypotheses from mitogenomic data and inferred the timing of the diversification of major lineages using fossil-calibrated molecular clock analysis to shed light on the biogeographic history of the family. Explicitly we examined the proposal that the most recent common ancestor of the Aemodogryllinae and Rhaphidophorinae existed in the Late Cretaceous, facilitating dispersal to North America and East Asia from Beringia (Kim et al., 2024).

MATERIALS AND METHODS

Taxon Sampling

In this study, we considered species representing six subfamilies of the Rhaphidophoridae from three major Northern Hemisphere geographical regions (North America, Europe, and Asia) and the Southern Hemisphere. Two datasets were generated. The larger taxon dataset (n = 117 species) was compiled to test the current generic classification system within the Aemodogryllinae and Rhaphidophorinae by incorporating data from as many species representatives as possible. We supplemented data available from previous phylogenetic studies of the Rhaphidophoridae (Aemodogryllinae and Rhaphidophorinae) from Asia (e.g., Zhu, Wang, et al., 2022; Zhu, Zhou, et al., 2022) with sampling from Bhutan, Oceania, and North America. Samples from Bhutan were collected during April 2022 under the authorization of research permit number 7008186946225A7A8C6D0B granted by the Ugyen Wangchuck Institute for Forestry Research and Training (UWIFoRT), Bhutan. Subsequent transfer to Massey University was conducted in accordance with a Material Transfer Agreement, reference number NBC/BRD/7/2022-2023/197. All samples were deposited in the Phoenix collection at Massey University, Palmerston North (MPN). We used DNA sequences (12S, 16S, COI, 28S, and 18S) from GenBank and the Barcode of Life Data System (BOLD) in conjunction with additional genetic data extracted from skim sequencing (Supplementary Table S1). This dataset will be referred to as the 117-taxa set.

For molecular clock analyses we compiled a dataset using the 13 protein-coding genes extracted from whole mitochondrial assemblies of 20 Rhaphidophoridae and 2 outgroup taxa (Table 1). This included representatives of diversity in Southern Hemisphere Macropathinae that constitute a sister to the clade that includes the Aemodogryllinae and Rhaphidophorinae in Asia and near Oceania. We generated new mitogenomic data for species in the subfamilies Rhaphidophorinae and Aemodogryllinae from Bhutan and the Solomon Islands and Ceuthophilinae from the United States of America. The Rhaphidophorinae and Aemodogryllinae were identified based on the descriptions provided in (Dawwrueng et al., 2020; Dorji et al., 2025; Gorochoy & Storozhenko, 1992, 2015, 2019). The Rhaphidophorinae specimen from Vangunu Island, Solomon Islands, was identified as belonging to the genus *Stonychophora* Karny upon comparing descriptions and high-resolution images of the type specimen *Stonychophora salomonensis* Willemse described from Aola, Solomon Islands (Karny, 1934; Willemse, 1942). We identified the specimen belonging to the Ceuthophilinae as a member of the genus *Ceuthophilus* Scudder (Scudder, 1861).

DNA Sequencing

We assembled entire mitochondrial genomes from DNA extractions without the need for PCR using skim sequencing techniques. For this, we carried out extraction of DNA from leg muscle using a salting-out protocol (Sunnucks & Hales, 1996; Trewick & Morgan-Richards, 2005). Genomic DNA samples were paired-end-sequenced through massive parallel, high-throughput sequencing on an Illumina HiSeq 2500 by Macrogen (Seoul, Republic of Korea) following fragmentation and indexing using the Illumina (San Diego, CA, USA) TruSeq Nano DNA kit. The resulting DNA reads with a mean fragment size of 150 base pairs (bp) were sorted and iteratively assembled in Geneious Prime v2022.2.2 (Kearse et al., 2012). Initial mapping reads from each sample used published annotated mtDNA genome data from GenBank under strict sensitivity settings to allow mapping with minimal gaps and ambiguity, generating novel consensus sequences (Koot et al., 2022; Vaux et al., 2017). Partial assemblies were remapped to close gaps and the resulting draft assembly remapped with raw sequence reads until all the alignment gaps were filled by extension with the new sequence data and ambiguities were resolved. Consensus sequences were checked for ambiguity and reading-frame anomalies and annotated by comparison with published examples, with submission to the MITOS server (Bernt et al., 2013). Reading frames, amino acid translation, and secondary structures were examined for protein-coding, ribosomal RNA (rRNA), and transfer RNA (tRNA) genes, respectively. Each of the 13 protein-coding genes were extracted and aligned separately using ClustalW aligner (default settings) before they were concatenated to give a final dataset 11,124 nucleotides in length.

Table 1. Rhaphidophoridae and sister Prothalangopsidae used in phylogenetic analysis of Asian subfamilies. DNA sequence data consist of 13 mitochondrial protein-coding genes, 22 tRNAs, and 2 rRNAs (14,800 bp). Taxa newly sequenced in this study are in bold, and their specimen codes are linked with specimens kept in the Phoenix Collection at Massey University, Palmerston North (MPN). * The names associated with GenBank accessions for *Diestrammena asynamora* and *Diestramima* sp. we hereafter refer to as *Tachycines asynamorus* and *Diestramima intermedia*, respectively, in accordance with (Qin, Jiang, et al., 2018; Zhu, Zhou, et al., 2022).

Family	Subfamily	Taxa	Country	Specimen Code	Genbank Accession No.	Author
Rhaphidophoridae	Aemodogryllinae	<i>Diestramima matern magna</i>	Pema Gats shel, Bhutan	MPN_CW5536	OR896621	This study
		<i>Diestramima tsongkhapa</i>	Trongsa, Bhutan	MPN_CW5525	OR896622	This study
		<i>Diestramima intermedia</i> *	China		KX057718	(Zhou et al., 2017)
		<i>Diestramima tibetensis</i>	China		KX057740	(Zhou et al., 2017)
		<i>Diestrammena</i> sp.	China		MT849270	(Yuan et al., 2021)
		<i>Diestrammena japonica</i>	Japan		MK347245	(Guan & Xu, 2025)
		<i>Tachycines asynamorus</i> *	China		KX057726	(Zhou et al., 2017)
		<i>Tachycines shuangcha</i>	China		OM993275	(Hong, 2025)
		<i>Tachycines zorzini</i>	China	MW322826	NC_057442	(Y. Wang et al., 2021)
		<i>Ceuthophilus</i> sp.	Moab Desert, USA	MPN_CW4347	OR880641	This study
	Rhaphidophorinae	<i>Stonychophora</i> sp.	Vangunu, Solomon Is.	MPN_ORT15	OR896624	This study
		<i>Rhaphidophora quadrispina</i>	China		OL450400	(Lu, 2025)
		<i>Rhaphidophora bicuspis</i>	Thimphu, Bhutan	MPN_CW5529	OR896623	This study
		<i>Rhaphidophora bhutanensis</i>	Pema Gats shel, Bhutan	MPN_CW5483	OR896625	This study
		<i>Rhaphidophora bilobata</i>	Trongsa, Bhutan	MPN_CW5545	OR896626	This study
		<i>Troglophilus neglectus</i>	Brje pri Kombu, Slovenia		EU938374	(Fenn et al., 2008)
	Macropathinae	<i>Macropathus</i> sp.	Waitomo, NZ	MPN_CW109	OR520204	(Dowle et al., 2024)
		<i>Talitropsis sedilotti</i>	Hawkes Bay, NZ	MPN_CW1830	OR551721	(Dowle et al., 2024)
		<i>Parvotettix domesticus</i>	Taronga, Tasmania	MPN_CW736	OR551716	(Dowle et al., 2024)
		<i>Spelaeiacris monslamiensis</i>	Hex River, South Africa	MPN_CW3801	OR551731	(Dowle et al., 2024)
Prothalangopsidae	Cyphoderrinae	<i>Cyphoderris monstrosa</i>			KM657332	(Song et al., 2015)
	Prothalangopsinae	<i>Tarragoilus diuturnus</i>			NC_021397	(Zhou et al., 2017)

Phylogenetic Analysis

For analysis of the 117-taxa set, we concatenated partial DNA sequences of three mitochondrial genes (12S, 16S, and COI) and two nuclear ribosomal genes (28S and 18S) to provide a total alignment length of 3314 bp. For phylogenetic inferences the data were partitioned based on gene type (ribosomal or protein-coding) and on codon position (for COI), creating seven partitions.

Phylogenetic reconstruction of five subfamilies within the Rhaphidophoridae used data from whole mitochondrial genomes. For these we extracted and concatenated the protein-coding genes and excluded the tRNA and rRNA genes based on the findings from previous studies on Orthoptera that found that the use of only protein-coding genes resulted in the most stable analyses and that inclusion of RNAs did not improve tree topology (Koot et al., 2022; Sun et al., 2017; Zhang et al., 2013). We explored the influence on topology and node support of different partition models using the Maximum Likelihood (ML) method on both nucleotides and amino acid sequence alignments. The final dataset for 20 taxa consisted of concatenated data for 13 protein-coding genes, and removing stop codons from each gene and trimming to equal alignment lengths yielded 13 partitions based on genes and 52 partitions based on genes plus codon positions.

For both datasets, we used Partition Finder 2 (Lanfear et al., 2017) implemented in IQ-TREE 2 v2.2.0 (Minh et al., 2020) to identify the optimal partition scheme and the most suitable models of DNA evolution under the Bayesian information criterion (Supplementary Table S2). The best partitioning schemes were used for reconstructions of phylogenetic relationships within the family. The ML analysis was performed in IQ-TREE 2 considering invariable sites, Gamma rate heterogeneity, and resampling strategy (Seo et al., 2005). Ultrafast Bootstrap (Hoang et al., 2018) and Sh-aLRT support values (Guindon et al., 2010) were calculated with 1000 replicates.

Divergence Time Estimate Analysis

We inferred the timing of lineage splitting among the rhaphidophorid subfamilies using BEAST2 v2.7.4 (Bouckaert et al., 2014). Since the only fossil record of Rhaphidophoridae is uninformative for dating the earliest splits, recent studies have relied on estimates of time of divergence using secondary fossil calibration points from a phylogenomic study of Orthoptera (e.g., (Kim et al., 2024; Zhu, Zhou, et al., 2022)). The reliability of node ages of subfamily lineages within the Rhaphidophoridae is uncertain due to under-representation of taxa (only 3

Rhaphidophoridae included in (Song et al., 2020)), which may result in significant skewing in molecular clock inferences. We also used a secondary fossil calibration but in conjunction with a fossil calibration constraining the sister lineage of Rhaphidophoridae. We calibrated our phylogeny using the fossils of Prothalangopsidae, which are considered to be close relatives of the raphidophorid family (Song et al., 2015, 2020). Positioning a calibration within the target group of analysis is essential for good molecular clock inference; therefore, we positioned a secondary fossil calibration to constrain the node age of the common ancestor of Macropathinae and (Aemodogryllinae + Rhaphidophorinae). Our secondary fossil calibration within the Rhaphidophoridae is based on an analysis that included the same Prothalangopsidae fossil calibration but with much wider outgroup sampling (nine taxa representing Anostomatidae, Stenopelmatidae, and Tettigoniidae) and included a well-dated geological constraint within their ingroup using endemic *Talitropsis* species of the Chatham Islands (Dowle et al., 2024). We constructed our phylogeny using mitochondrial genomes. We examined the effects on divergence times, model convergence, and Effective Sampling Size (ESS) scores of applying different distribution priors (normal, log-normal, and exponential) (Ho & Phillips, 2009). We found that a normal distribution prior on both the fossils outside the raphidophorids and on a secondary node constraining Macropathinae + Aemodogryllinae + Rhaphidophorinae gave the optimal result, and these were used for the final analysis (Table 2).

1. Fossils belonging to the genus *Aboilus* Martynov (201.3–157.3 million years ago [Mya]) are the oldest definitive Prothalangopsidae known (Lin & Huang, 2006; Wang et al., 2015). These are well-recognized fossils previously used in divergence dating analysis of Orthoptera (Song et al., 2015; Zhou et al., 2017). *Aboilus* consists of 20 extinct taxa (Cigliano et al., 2023), with fossils from the Jurassic and Cretaceous. We constrained two commonly used extant species of Prothalangopsidae in our molecular analyses (represented by *Cyphoderris monstrosa* Uhler and *Tarragoilus diuturnus* Gorochov) under a normal distribution prior with the minimum fossil age as a minimum soft bound (157.3 Mya) and 192 Mya as a maximum soft bound.
2. As a secondary fossil calibration point, we constrained our analysis using a recent phylogenomic study of Southern Hemisphere raphidophorids calibrated with a Prothalangopsidae fossil (*Aboilus*) and a recent geological constraint (Dowle et al., 2024). We used 160 Mya as a mean age, with 95% Highest Posterior Density (HPD) values as soft minimum and maximum bounds at the node of Macropathinae and Aemodogryllinae + Rhaphidophorinae.

The BEAST input file was generated using BEAUti2 v2.7.5 (Drummond et al., 2012) by implementing parameters for molecular clock models, trees, and fossil priors. As in the ML phylogeny, we used optimal partitioning by genes in the concatenated protein-coding mitochondrial DNA dataset by linking clock and tree models for three final substitution models. We implemented a GTR model with non-standard substitution models by allowing parameter linking. Fossil-calibrated analyses were run under an uncorrelated optimized relaxed clock model with the birth–death process as a tree prior.

For comparison we also calibrated dating analyses of the same DNA dataset using two node age estimates derived from a previous study that relied on the paleogeographic inference of the timing of ancient land distributions (referred to here as ‘landscape’ calibration). This approach makes the assumption that continental drift was the primary influence on lineage splitting in the Rhaphidophoridae (Allegrucci & Sbordoni, 2019). Thus, the split between the North American Ceuthophilinae and the European Troglophilinae was set at 68.4 Mya with 95% HPD bounds of 46.3–99.3, and the ancestor of Aemodogryllinae and Rhaphidophorinae with Macropathinae was set to 117 Mya with 95% HPD bounds of 105–130. We enforced similar settings as above in BEAUti2 using a relaxed molecular clock and the birth–death process as a tree prior. We used 95% HPD values of these node ages as soft minimum and maximum bounds in a normal prior distribution around the secondary landscape calibration points.

For both calibration approaches we enforced monophyly constraints on each subfamily for consistency in topologies of molecular clock dating trees with the ML tree topology from 13 protein-coding genes. Initially, to assess the convergence of models using different distribution priors, we conducted BEAST2 analysis of 10 million generations. After considering analysis convergence and evaluation of Effective Sampling Sizes, final BEAST2 runs of 100 million generations with sampling every 1000 generations were performed. Tracer v1.7 (Rambaut, 2018) was used to inspect the results, and ESS statistics were investigated to check the fit of the models. We considered ESS values greater than 250 sufficient for the analyses to be informative after discarding 10% of the run as burn-in. Maximum clade credibility trees with median heights were generated in TreeAnnotator v2.7.1 (Drummond & Rambaut, 2007) after removing fossils and were visualized in FigTree v1.4.4 (Rambaut, 2018).

Table 2. Internal node estimates (most recent common ancestors (MRCAs)) of subfamilies of Northern Hemisphere camel crickets (Rhopidophoridae). Node ages inferred from primary calibration using an orthopteran fossil and secondary fossil-calibrated node ages (above) and (below) from secondary landscape calibration based on estimated inferred paleogeographic history (Allegrucci & Sbordoni, 2019; Koot et al., 2020). Median ages and 95% Highest Posterior Density credibility intervals are in millions of years ago (Mya). Abbreviation used for subfamilies: Ceuthophilinae (Ceu); Troglophilinae (Tro); Macropathinae (Mac); Rhaphidophorinae (Rha); and Aemodogryllinae (Aem). Prior distribution models for fossil (F) and secondary (S) calibrations were U = Uniform, LN = Log-Normal, N = Normal, and E = Exponential. Number of generations (N. gen.) in millions. Effective sampling sizes are provided for posteriors, tree likelihoods, and priors after removing 10–40% as burn-in.

BEAST RUN	Priors F/S	Posterior Tree Likelihood	Prior	Rhaphs. MRCA	95% HPD	Ceu and Tro MRCA	95% HPD	Rha and Aem MRCA	95%HPD	Fossil Treatment: Aboilus (201-157 Mya)	Sec. Treatment: Mac/Aem + Rha Node Age (140-180 Mya)	
1	LN/N	411	327	368	188.7	166.6; 210.7	153.4	131; 178.7	137.6	121.5; 154.9	Hard min = 157; mean = 172; 97.5% max = 222	2.5% min = 140; mean = 160; 97.5 max = 180
2	N/N	1109	846	1819	188.8	165.7; 211.3	153.6	129.8; 178	138.1	120.7; 154.9	2.5% min = 152; mean = 172; 97.5% max = 192	2.5% min = 140; mean = 160; 97.5 max = 180
3	E/E	1019	775	1234	188.7	159.2; 226.8	153.7	124.7; 189.7	137.6	115.2; 166.1	Hard min = 157; mean = 172; 97.5% max = 212	Hard min = 140; mean = 160; 97.5% max = 214
4	LN/E	945	675	1173	188.6	156.4; 227.2	153.6	126.1; 189.7	135.3	113.2; 170.6	Hard min = 157; mean = 172; 97.5% max = 222	Hard min = 140; mean = 160; 97.5% max = 214
5	N/N	10278	7661	20,075	188.7	166.7; 211.9	153.6	130; 178.5	138.1	121.1;155	2.5% min = 152; mean = 172; 97.5% max = 192	2.5% min = 140; mean = 160 97.5 max = 180
BEAST RUN	Prior S/S	Posterior Tree Likelihood	Prior	Rhaphs. MRCA	95% HPD	Ceu and Tro MRCA	95% HPD	Rha and Aem MRCA	95% HPD	SEC. Node Age Treatment: Ceu and Tro (99.3-46.3 Mya)	Sec. Node Age Treatment: Mac/Aem + Rha Node Age (130-105 Mya)	
1	N	707	663	1245	109.5	98.0; 122.4	69.9	65.8; 73.6	88.5	77.6; 100.8	2.5% min = 48.9; mean = 68.5; 97.5% max = 88	2.5% min = 105; mean = 117; 97.5% max = 129
2	E	635	511.1	1266	113	105.3; 127.7	85.4	69.8; 102.2	90.4	81.4; 103.8	Hard min = 56; mean = 68.5; 97.5% max = 102	Hard min = 105; mean = 117; 97.5% max = 149
3	U	776	626.9	1388.3	121	106.6; 136.5	94.5	76.2; 115.9	95.5	84.1; 108.5	Bounds: min = 46; mean = 72.5; max = 99	Bounds: min = 105; mean = 118; max = 130
4	N	8860	8032	10,664	109.8	97.7; 122.1	69.8	66; 73.6	88.3	77.1; 100.4	2.5% min = 48.9; mean = 68.5; 97.5% max = 88	2.5% min = 105; mean = 117; 97.5% max = 129

RESULTS

Systematics

The ML tree for the set of 117-taxa resulted in six well-supported clades congruent with recognized subfamilies of the Rhaphidophoridae. All the clades were supported by high statistical bootstrap values > 90 (Figure 2). Although diverse in terms of body size and color patterns, most raphidophorid genera are recognized by a few traits that typically include apical spines on legs and features of male terminalia. Nevertheless, our phylogenetic hypothesis was mostly concordant with the current taxonomy. Within the subfamily Aemodogryllinae, four of the six genera sampled formed separate monophyletic clades. The notable exception to this was the placement of six taxa in the genera *Diestrammena* Brunner von Wattenwyl and *Tachycines* Adelung in three separate paraphyletic clades: (a) *Tachycines asynamorus* (Adelung) with *Tachycines shuangcha* Feng, Huang & Luo having a close relationship to *Gymnaetoides* Qin, Liu & Li; (b) *Diestrammena japonica* Blatchley with *Diestrammena fengyangshanica*; and (c) *Tachycines zorzini* (Rampini & Di Russo) with *Diestrammena* sp. (Figure 2).

Rhaphidophoridae Mitogenomic Data

New mitochondrial genomes were assembled and annotated for seven raphidophorids. For all sampled taxa (Table 1), gene order was typical of mitochondrial genomes already documented for Rhaphidophoridae, comprising the expected complement of 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and a repeat region (putative control region) (Supplementary Figure S1) (Dowle et al., 2024). The final length of 13 concatenated protein-coding genes varied from 11,208 bp (MPN_CW4347) to 11,231 bp (MPN_CW5536 and MPN_CW5525), with truncated AT stop codons in COII, ND4, and ND5 in some sampled taxa (Supplementary Table S3). Assembled mitochondrial genomes, excluding the putative control region that lies between the small rRNA and tRNAs adjacent to ND2, ranged in size from 14,718 bp (MPN_ORT15) to 14,885 bp (MPN_CW5529).

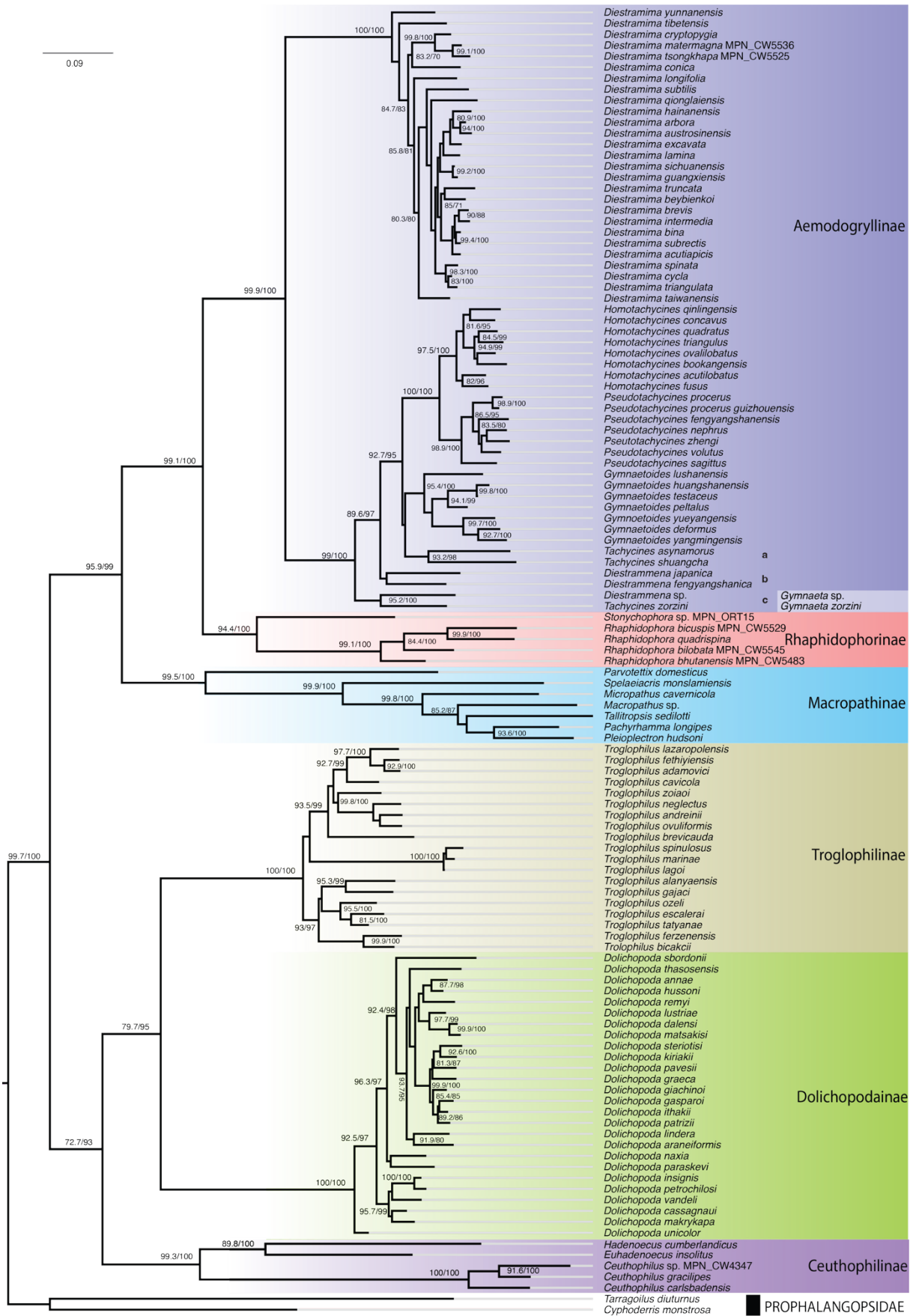


Figure 2. Phylogeny of camel crickets (Rhaphidophoridae) inferred from DNA sequences. ML analysis based on concatenated alignment of partial mitochondrial (COI, 12S, and 16S) and nuclear rRNA (18S and 28S) gene sequences (total: 3314 bp). ML bootstrap proportions > 90% of 1000 replicates are shown at nodes along with respective Sh-aLRT values. Labels a, b, and c in the tree (in subfamily Aemodogryllinae) indicate three monophyletic lineages comprising the paraphyletic genera *Diestrammena* and *Tachycines*. Resurrection of *Gymnaeta* for lineage c would render lineages a (*Tachycines*) and b (*Diestrammena*) monophyletic.

Phylogenetic Analysis and Time Calibration

Phylogenetic analysis of the concatenated alignment of 13 mitochondrial protein-coding genes (11,124 bp) yielded a resolved tree topology with high node bootstrap support (99 or 100%) (Supplementary Figure S2). Topologies from different partition and model schemes on nucleotide and amino acid alignments were congruent and without significant differences in node support values, with a gene concordance factor >45 (Supplementary Figures S3–S5). The topology was found to be consistent with the current classification of rhaphidophorid subfamilies. Despite the smaller number of taxa representing the subfamily Aemodogryllinae, the paraphyletic nature of the genera *Diestrammena* and *Tachycines* was consistent with our findings from the analysis of a combination of partial gene sequences for 117-taxa. *Stonychophora* from the Vangunu Island, Solomon Islands (MPN_ORT15), were grouped within the Asian subfamily Rhaphidophorinae but on a long branch.

Following standard practice (Barba-Montoya et al., 2021; Koot et al., 2020; Saladin et al., 2017), initial fossil calibration and secondary landscape calibration analyses were performed using 10 million generations for each under different combinations of prior distributions to assess the effects on convergence with reference to Effective Sampling Size (ESS) statistics examined in Tracer. During this process, it was recognized that all the analyses started to converge between 1 and 4 million generations, with stable node ages and good ESS values (>250) for all the priors (after removing 10–40% as burn-in), with the exception of secondary landscape calibration that showed varying node ages and 95% HPD intervals. Analysis using a combination of log-normal and exponential priors yielded a wide range of upper and lower bounds for all node ages. For the final analysis, normal priors for both the calibration points (fossil and secondary fossil calibration) were found to be optimal based on the evaluation of ESS statistics (Table 2, Beast run 5). For secondary landscape calibration, uniform and exponential distribution priors produced slightly higher median ages compared to results with a normal prior and very wide ranges of minimum and maximum 95% HPD intervals for the node ages of Troglophilinae and

Ceuthophilinae. Final analysis of secondary landscape calibration was therefore performed using normal prior distributions (Table 2, Beast run 4).

Our fossil-calibrated molecular clock analysis estimated the origin of Northern Hemisphere Rhaphidophoridae subfamilies during the Jurassic and Early Cretaceous periods of the Mesozoic Era (Figure 3). The earliest common ancestor of the Rhaphidophoridae we sampled is estimated to have lived during the Early Jurassic, 188.7 Mya (Table 2). We estimated that the split between the North American Ceuthophilinae and Mediterranean Troglophilinae (~153 Mya) and Asiatic lineages from Southern Hemisphere Macropathinae (~164 Mya) also occurred during the Mesozoic. The Aemodogryllinae and Rhaphidophorinae diverged during the Cretaceous (~138 Mya). The most recent common ancestor within the Rhaphidophorinae diverged at about 105 Mya in the Early Cretaceous, represented by the node between *Stonychophora* from Vangunu Island, Solomon Islands (MPN_OR15), and the remaining Asian taxa diverged throughout the Cenozoic Era. The common ancestor of the sampled Aemodogryllinae taxa was estimated at 110 Mya.

In contrast to the fossil-calibrated analysis, our secondary landscape calibration of the phylogeny based on inferences from landscape history suggests significantly younger node ages with narrower credibility intervals (Table 2). The estimation of the most recent common ancestor for Rhaphidophoridae indicates a divergence that occurred approximately 110 Mya during the Early Cretaceous. This divergence was followed by the separation of North American and Mediterranean taxa around 70 Mya and the divergence of Asiatic taxa around 108 Mya from the Macropathinae. Within the Asiatic lineages, the common ancestor of the Aemodogryllinae and Rhaphidophorinae lived about 88 Mya. The divergence of the most recent common ancestor of the Pacific taxon and the Asian taxa was estimated to have occurred at 67.8 Mya, while the divergence of the Aemodogryllinae was estimated at 70.5 Mya (Supplementary Figure S6).

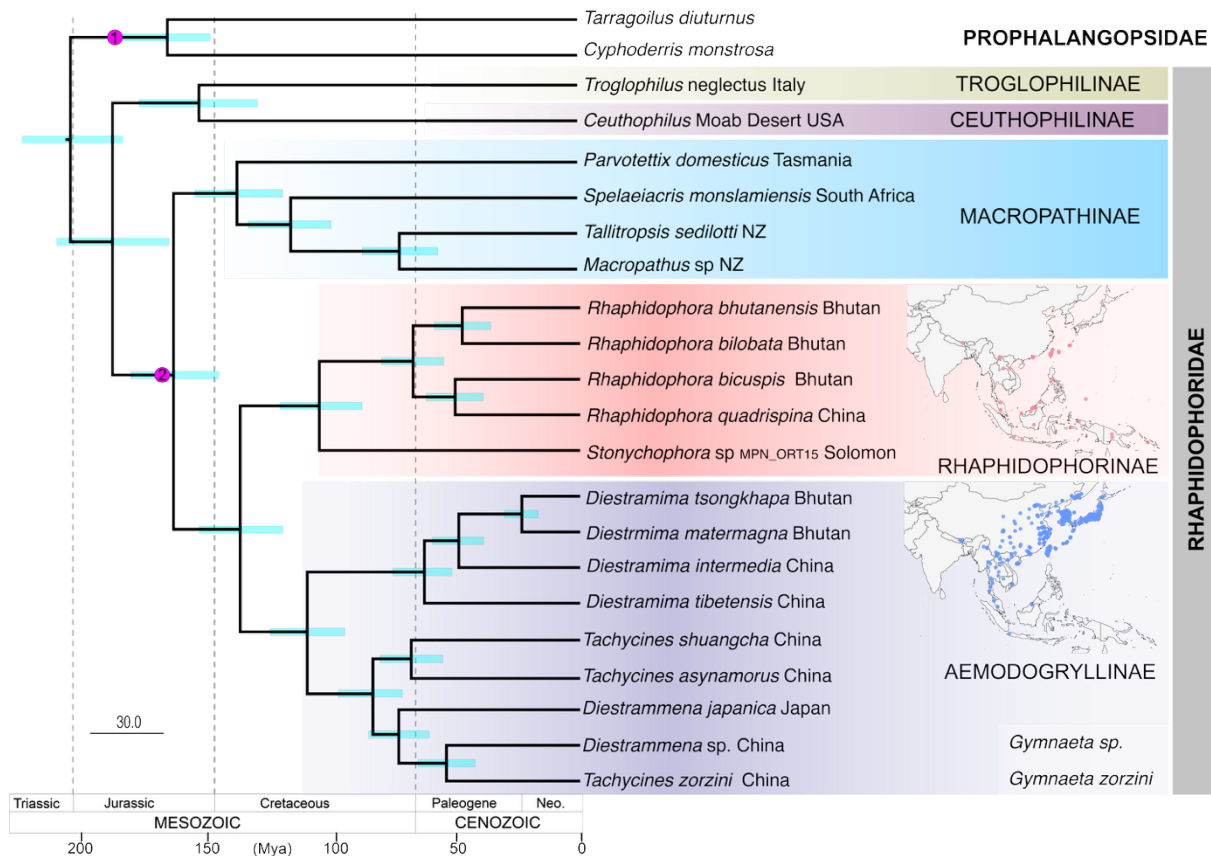


Figure 3. Chronogram of Rhaphidophoridae focusing on Asian lineages inferred by Bayesian analysis of 13 protein-coding genes calibrated with fossil and secondary fossil calibration points and a relaxed molecular clock. Calibration (1) fossil *Aboilus* (Prophalangopsidae) at approximately 172 Mya and (2) secondary fossil calibration point at 160 Mya as node ages of Macropathinae and Aemodogryllinae + Rhaphidophorinae (Table 2). Turquoise bars at nodes indicate 95% HPD credibility intervals. Inset maps indicate known locality records for the two subfamilies Rhaphidophorinae and Aemodogryllinae.

DISCUSSION

Systematics

Our phylogenetic analysis of 117 Rhaphidophoridae found an excellent concordance between the taxonomy and evolutionary relationships, despite morphological conservation within this family. The placement of only six of the included taxa within *Diestrammena* and *Tachycines* constitutes an exception to the general observation of monophyletic genera. There is inconsistency between the phylogenetic relationships among six *Diestrammena* and *Tachycines* taxa inferred from DNA sequence data with the current classification scheme of two subgenera based on morphology (Karny, 1934; Qin, Jiang, et al., 2018). Discrepancies in the assignment of subgenera have also been reported for other genera (e.g., *Diestrammena* Storozhenko) in the

same subfamily (Zhu, Zhou, et al., 2022). Adelung (1902) erected the genus *Tachycines*. Since then, the taxonomic placement of taxa associated with *Tachycines* Adelung and *Diestrammena* Brunner von Wattenwyl and with *Gymnaeta* Adelung has been repeatedly modified with the use of the additional subgenus rank (Furukawa, 1933; Gorochov & Storozhenko, 1992; Karny, 1934; Qin, Jiang, et al., 2018; Zhang & Liu, 2009). The lack of monophyly identified by our analysis of molecular data suggests that a third genus needs to be recognized to resolve the current inconsistencies (Figure 2, clades a, b, and c). This can be achieved by resurrection of the genus *Gymnaeta* Adelung and by revising *Tachycines zorzini* (Rampini & Di Russo) as *Gymnaeta zorzini* and *Diestrammena* sp. (GenBank accession: MT849270) as *Gymnaeta* sp., while maintaining the monophyly of *Tachycines* and *Diestrammena*.

Lineage Age and Origin

Some of the uncertainties of molecular clock analysis, including substitution rate variation, are integrated into molecular dating within the Bayesian framework (Inoue et al., 2010), but the scarcity of appropriate fossil material and the potential for uncertain interpretation of their systematic relationships is inevitable. We showed that the prior distribution models (normal, log-normal, or exponential) used for our calibrations of molecular clock analyses did not significantly impact our estimates of the timing of common ancestors. However, our fossil calibration (in the outgroup) and secondary fossil calibration node (in the ingroup) are not entirely independent of one another and therefore might result in the appearance of greater accuracy than is realistic in our molecular clock analysis. The universal uncertainties from incomplete sampling that results from both extinction and lack of specimens for DNA sequencing must also be considered (Crisp et al., 2011; Grandcolas et al., 2014). Our analysis included new samples representing the subfamilies Aemodogryllinae and Rhaphidophorinae from a wider geographic range than previously considered, including the Kingdom of Bhutan in the Himalaya. Better representation of the diversity within these lineages should improve our estimates of the age of their most recent common ancestor. However, the poor sampling from the Pacific Islands and the lack of samples from India, where divergent lineages may exist, remain a major challenge. Therefore, we need to treat all date estimates as hypotheses that require further testing.

If the calibration of our data-rich molecular clock analysis is realistic, then the orthopteran family of camel crickets (Rhaphidophoridae) had a common ancestor before Pangea broke into the supercontinents of Gondwana and Laurasia (~180 Mya). The Jurassic was a major period of

continental movement and would appear to have coincided with the divergence of the Mediterranean and North American subfamilies from the Asian and southern subfamilies. However, the confidence intervals around divergence estimates based on fossil calibrations are such that it will be difficult to test vicariant hypotheses based on events that took place so long ago. If the orthopteran fossils from Triassic and Jurassic deposits used for time calibration have been correctly identified, then the age of the raphidophorid family is much older than inferred based on an assumption of landscape vicariance (Allegrucci & Sbordoni, 2019) and calibration based on secondary constraints derived from a phylogenomic study of Orthoptera (Kim et al., 2024). Specifically, the most recent common ancestor of the European lineages (Troglophilinae and Dolichopodainae) and the North American lineage (Ceuthophilinae) seems to have existed before the supercontinent Laurasia broke up to give rise to the basal rocks of Europe, North America, and much of Asia.

Divergence of the Raphidophoridae family from a common ancestor during the Late Triassic and Jurassic periods is compatible with the divergence dates of Raphidophorids obtained from fossil-calibrated phylogeny studies of Ensifera (Dowle et al., 2024; Song et al., 2015, 2020; Zhu, Zhou, et al., 2022). The increase in subfamily diversity of Raphidophoridae is also consistent with the Jurassic-to-Early Cretaceous peak in insect diversity (Clapham et al., 2016; Schachat et al., 2019), which is in part explained by a warmer climate and the Early Cretaceous radiation of angiosperms (Jud, 2015; Silvestro et al., 2021). We constrained our molecular clock analysis so that the Southern Hemisphere Macropathinae shared a common ancestor with Asiatic Aemodogryllinae and Raphidophorinae about 160 Mya, which agrees with a hypothesis based on morphology (Ander, 1939; Karny, 1934).

Divergence times inferred from our alternative analysis using secondary landscape calibrations were significantly younger than those estimated with our fossil calibration. Inconsistency in estimates derived from geophysical models of landscape changes have previously been observed (Beasley-Hall et al., 2018; Kim et al., 2024), and ambiguity about the timing and biological influence of paleoenvironments such as Beringia (e.g., (Brikiatis, 2014; Wen et al., 2016)) suggests that they are probably inappropriate for constraining phylogeny. The spatial distribution of lineage diversity in the Macropathinae also shows that the location of extant representatives of deep lineages does not indicate the ancestral location (Crisp et al., 2011; Grandcolas et al., 2014; Trewick & Morgan-Richards, 2016).

We inferred that a common ancestor of the two Asian subfamilies Aemodogryllinae and Rhaphidophorinae existed during the Late Jurassic/Early Cretaceous (155–121 Mya), when Gondwana and Laurasia were breaking up, and prior to the Late Cretaceous (110–65 Mya) Beringia land connection of North America and Asia. Moreover, stratigraphic evidence indicates that subduction of the Indian tectonic plate under Asia began about 61 Mya (An et al., 2021), with the subsequent formation of connecting land masses through continental collision about 45 Mya (Pusok & Stegman, 2020). In our analysis, the earliest diversification of representative Aemodogryllinae and Rhaphidophorinae from Bhutan, Southeast China, and Japan occurred before the Tertiary contact of the Indian subcontinent and Eurasian tectonic plates (White & Lister, 2012). The estimated diversification of *Diestramima* starting at about 63 Mya is inconsistent with the timing of the Himalayan emergence and the associated change in weather patterns thought to have influenced the diversification of this genus in Southeast China (Zhu, Zhou, et al., 2022).

Our sample of *Stonychophora* from the Solomon Islands is at the southernmost end of the known range of the Rhaphidophorinae and brings this subfamily geographically close to the Macropathinae that span the Southern Hemisphere (Figure 1). In the current analysis *Stonychophora* is sister to *Rhaphidophora* species sampled from Bhutan, and their common ancestor was estimated to have existed about 106 Mya. We know through radiometric dating that the Solomon Islands archipelago started to form from volcanic activity 45–40 Mya (Hall, 2002), and Vangunu Island, where the specimen was collected, formed from more recent Plio-Pleistocene volcanics (Mann et al., 1998). This indicates a complex biogeographic history of *Stonychophora* in the archipelago, Papua New Guinea, and through the Malay Archipelago—a region long recognized as a biogeographic nexus (Simpson, 1977). The establishment of such island biotas requires colonization from dispersal over water (Dowle et al., 2024; Grandcolas & Trewick, 2016; Keppel et al., 2009), which could obscure any signal of the spatial origins of subfamily lineages. The Solomon Islands archipelago spans a large area, with islands of various ages, so further sampling in this region is needed to interpret raphidophorid diversity in the region and its evolutionary history in relation to other Rhaphidophorinae in Southeast Asia. Nevertheless, it appears that the ancestral connection of these lineages to the Asian continent was via Southeast Asian, through the Western Pacific, rather than through India or Beringia.

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SUPPLEMENTARY INFORMATION

Table S1. Rhaphidophorid (117-taxa) included in this study with location details and GenBank accession numbers. Approximately 3,314 bp DNA sequence data consists of 2071 mitochondrial DNA (COI=1197, 12S=368 and 16S=504) and 1233 Nuclear rRNA (551 18S and 682 28S). Additional taxa newly sequenced for this study are in bold. Two representatives of Prophalangopsidae (*Cyphoderris monstrosa* and *Tarragoilus diuturnus*) were employed as an outgroup.

Sub Family	Taxa	Code	Location	GenBank accession number					Reference
				COI	12S	16S	18S	28S	
Aemodogryllinae	<i>Diestramima tsongkhapa</i>	MPN_CW5525	Bhutan	OR896622 (complete mt genome)					This study
	<i>Diestramima matern magna</i>	MPN_CW5536	Bhutan	OR896621 (complete mt genome)					This study
	<i>D. acutiapicis</i>	DI037GZ	Lianhuaping, Leigong Mountain, Guizhou, China	MW961044	MW962019	MW961784	MW961666	MW961902	Zhu et al. 2022
	<i>D. arbora</i>	DI068GX	Shiwanda Mountain, Shangsi, Guangxi, China	MW961064	MW962039	MW961804	MW961686	MW961922	Zhu et al. 2022
	<i>D. austrosinensis</i>	DI033ZJ	Pan'an County, Jinhua City, Zhejiang, China	MW961042	MW962017	MW961782	MW961664	MW961900	Zhu et al. 2022
	<i>D. beybienkoi</i>	DI004GZ	Mayang River, Tongren City, Guizhou, China	MW961025	MW962000	MW961765	MW961647	MW961883	Zhu et al. 2022
	<i>D. bina</i>	DI792HN	Mang Mountain, Yizhang County, Hunan, China	MW961119	MW962091	MW961859	MW961741	MW961977	Zhu et al. 2022
	<i>D. brevis</i>	DI027ZJ	Tianmu Mountain, Zhejiang, China	MW961038	MW962013	MW961778	MW961725	MW961961	Zhu et al. 2022
	<i>D. conica</i>	DI060YN	Baihualing, Boshan City, Yunnan, China	MW961057	MW962032	MW961797	MW961679	MW961915	Zhu et al. 2022
	<i>D. cryptopygia</i>	DI062YN	Tongbiguan Village, Yingjiang County, Yunnan, China	MW961058	MW962033	MW961798	MW961680	MW961916	Zhu et al. 2022
	<i>D. cyclo</i>	DI040SC	Wanniansi, Emei Mountain, Sichuan, China	MW961047	MW962022	MW961787	MW961669	MW961905	Zhu et al. 2022
	<i>D. excavata</i>	DI639GX	Huaping, Longsheng, Guangxi, China	MW961076	MW962048	MW961816	MW961698	MW961934	Zhu et al. 2022
	<i>D. furcata</i>	DI807GX	Baihe Napo, Guangxi, China	MW961132	MW962103	MW961872	MW961754	MW961989	Zhu et al. 2022
	<i>D. guangxiensis</i>	DI687GX	Daming Mountain, Nanning City, Guangxi, China	MW961096	MW962068	MW961836	MW961718	MW961954	Zhu et al. 2022
	<i>D. hainanensis</i>	DI631HN	Wuzhi Mountain, Hainan, China	MW961071	MW962043	MW961811	MW961693	MW961929	Zhu et al. 2022
	<i>D. intermedia</i>	DI001SC	Hongkou Village, Dujiangyan City, Sichuan, China	MW961024	MW961999	MW961764	MW961646	MW961882	Zhu et al. 2022
	<i>D. lamina</i>	DI066GX	Nonggang, Longzhou County, Guangxi, China	MW961062	MW962037	MW961802	MW961684	MW961920	Zhu et al. 2022
	<i>D. longifolia</i>	DI801YN	Maandi Village, Jinping, Yunnan, China	MW961128	MW962099	MW961868	MW961750	MW961985	Zhu et al. 2022
	<i>D. qionglaiensis</i>	DI657SC	Nanmuxi, Qionglai City, Sichuan, China	MW961094	MW962066	MW961834	MW961716	MW961952	Zhu et al. 2022

Table S1 (cont.).

Sub Family	Taxa	Code	Location	GenBank accession number					Reference
				COI	12S	16S	18S	28S	
Aemodogryllinae	<i>D. sichuanensis</i>	DI794SC	Huagaoxi, Xuyong County, Sichuan, China	MW961121	MW962093	MW961861	MW961743		Zhu et al. 2022
	<i>D. spinata</i>	DI046YN	Gulinjing Village, Maguan County, Yunnan, China	MW961050	MW962025	MW961790	MW961672	MW961908	Zhu et al. 2022
	<i>D. subrectis</i>	DI045GX	Dayao Mountain, Jiuxiu County, Guangxi, China	MW961049	MW962024	MW961789	MW961726	MW961962	Zhu et al. 2022
	<i>D. subtilis</i>	DI029YN	Wenlong Village, Pu'er City, Yunnan, China	MW961040	MW962015	MW961780	MW961662	MW961898	Zhu et al. 2022
	<i>D. taiwanensis</i>	DI083TW	Jilong City, Taiwan	MW961067	MW962042	MW961807	MW961689	MW961925	Zhu et al. 2022
	<i>D. tibetensis</i>	DI070XZ	80K, Medog County, Xizang, China	MW961066	MW962041	MW961806	MW961688	MW961924	Zhu et al. 2022
	<i>D. triangulata</i>	DI028GZ	Leigong Mountain, Guizhou, China	MW961039	MW962014	MW961779	MW961661	MW961897	Zhu et al. 2022
	<i>D. truncata</i>	DI036GX	Dayao Mountain, Jiuxiu County, Guangxi, China	MW961043	MW962018	MW961783	MW961665	MW961901	Zhu et al. 2022
	<i>D. yunnanensis</i>	DI809YN	Menglun Botanical Garden, Yunnan, China	MW961134	MW962105	MW961874	MW961756	MW961991	Zhu et al. 2022
	<i>Diestrammena</i> sp.		China	MT849270					Yuan et al. 2020
	<i>D. asynamora</i>		China	KX057726				KR903068	Zhou et al. 2017; Chintauan-Marquier al. 2016
	<i>D. fengyangshanica</i>	DD199ZJ	Zhejiang, Longquan, Fengyangshan, China	ON129823	ON146711	ON146820	ON146765	ON146655	Zhu et al. 2022
	<i>D. japanica</i>		Japan	MK347245					Guan, & Xu, 2018
	<i>Gymnaetoides deformus</i>	PT127ZJ	Zhejiang, Linan, Tianmu Mountain, China	ON129846	ON146678	ON146788	ON146734	ON146622	Zhu et al. 2022
	<i>G. huangshanensis</i>	GY879AH	Anhui, Huang Mountain, Tangkou, China	ON129833	ON146665	ON146775	ON146721	ON146609	Zhu et al. 2022
	<i>G. lushanensis</i>	GY113JX	Jiangxi, Lu Mountain, Guling, China	ON129825	ON146657	ON146767	ON146713	ON146601	Zhu et al. 2022
	<i>G. petalus</i>	GY112AH	Anhui, Chizhou City, Shitai County, Gongxi Village, Du hamlet, Penglaixian Cave, China	ON129824	ON146656	ON146766	ON146712	ON146600	Zhu et al. 2022
	<i>G. testaceus</i>	GY115ZJ	Zhejiang, Linan, Tianmu Mountain, China	ON129827	ON146659	ON146769	ON146715	ON146603	Zhu et al. 2022
	<i>G. yangmingensis</i>	GY733HN	Hunan, Shuangpai, Yangmingshan, China	ON129830	ON146662	ON146772	ON146718	ON146606	Zhu et al. 2022
	<i>G. yueyangensis</i>	PT120JX	Jiangxi, Lu Mountain, Guling, China	ON129841	ON146673	ON146783	ON146729	ON146617	Zhu et al. 2022
	<i>Homotachycines acutilobatus</i>	GY731HB	Hubei, Shennongjia, Huangbaiqian, China	ON129829	ON146661	ON146771	ON146717	ON146605	Zhu et al. 2022

Table S1 (cont.).

Sub Family	Taxa	Code	Location	GenBank accession number					Reference
				COI	12S	16S	18S	28S	
Aemodogryllinae	<i>H. baokangensis</i>	PT824HB	Hubei, Baokang, Houping, China	ON129864	ON146696	ON146806	ON146750	ON146640	Zhu et al. 2022
	<i>H. concavus</i>	PT830HN	Henan, Luoyang, Longyuwan, China	ON129867	ON146699	ON146809	ON146753	ON146643	Zhu et al. 2022
	<i>H. fusus</i>	PT663SX	Shaanxi, Yang County, Huayang, China	ON129849	ON146681	ON146791	ON146737	ON146625	Zhu et al. 2022
	<i>H. ovalilobatus</i>	PT746HB	Hubei, Shennongjia, Huangbaiqian, China	ON129858	ON146690	ON146800	ON146744	ON146634	Zhu et al. 2022
	<i>H. qinlingensis</i>	PT662SX	Shaanxi, Yang County, Huayang, China	ON129848	ON146680	ON146790	ON146736	ON146624	Zhu et al. 2022
	<i>H. quadratus</i>	PT124HB	Hubei, Shennongjia, Banbiyan, China	ON129845	ON146677	ON146787	ON146733	ON146621	Zhu et al. 2022
	<i>H. triangulus</i>	PT122HB	Hubei, Shennongjia, Maohu, China	ON129843	ON146675	ON146785	ON146731	ON146619	Zhu et al. 2022
	<i>Pseudotachycines fengyangshanensis</i>	PT744ZJ	Zhejiang, Longquan, Fengyangshan, China	ON129857	ON146689	ON146799	ON146743	ON146633	Zhu et al. 2022
	<i>P. nephrus</i>	PT742ZJ	Zhejiang, Linan, Qingliangfeng, China	ON129855	ON146687	ON146797		ON146631	Zhu et al. 2022
	<i>P. procerus</i>	PT822AH	Anhui, Yuexi, Yaoluoping, China	ON129862	ON146694	ON146804	ON146748	ON146638	Zhu et al. 2022
	<i>P. procerus guizhouensis</i>	PT110GZ	Guizhou, Suiyang, Kuankuoshui, China	ON129837	ON146669	ON146779	ON146725	ON146613	Zhu et al. 2022
	<i>P. sagittus</i>	PT667YN	Yunnan, Yiliang, Chaotianma, China	ON129850	ON146682	ON146792	ON146738	ON146626	Zhu et al. 2022
	<i>P. volutus</i>	PT108ZJ	Zhejiang, Linan, Tianmu Mountain, China	ON129835	ON146667	ON146777	ON146723	ON146611	Zhu et al. 2022
	<i>P. zhengi</i>	PT118JX	Jiangxi, Lu Mountain, Guling, China	ON129839	ON146671	ON146781	ON146727	ON146615	Zhu et al. 2022
	<i>Tachycines shuangcha</i>		China		OM993275.1				Hang 2020
	<i>T. zorzini</i>	MW322826	China	NC_057442					Y. Wang et al. 2021
Ceuthophilinae	<i>Ceuthophilus</i> sp.	MPN_CW4347	Moab Desert, USA	OR880641 (complete mt genome)					This study
	<i>C. carlsbadensis</i>			KU377020	Z97597	Z97613	Z97563		Flook et al. 1999; Weckstein et al. 2016
	<i>C. gracilipes</i>	CEU/GRA	Hamden, CT, USA	AY793593	MK993663	AY793561		MK993657	Alleggrucci et al. 2019; Alleggrucci et al. 2005
	<i>Euhadenoecus insolitus</i>	INDe	Indian Grave Point Cave, The Kalb Co., TN, USA	AY793591	F216948	AY793563	MK993679	EF217005	Alleggrucci et al. 2019; Alleggrucci et al. 2005
	<i>Hadenoecus cumberlandicus</i>	BATH	at Cave, Carter Cave State Park, Carter Co., KY, USA	AY793592	EF216947	AY793562	MK993680	EF217004	Alleggrucci et al. 2019; Alleggrucci et al. 2005

Table S1 (cont.).

Sub Family	Taxa	Code	Location	GenBank accession number					Reference
				COI	12S	16S	18S	28S	
Dolichopodainae	<i>Dolichopoda annae</i>	TEM	Aghlia Paraskevi Cave, Tembi Valley, Larissa, Greece	EU887894	EU887846	EU887861	MK993723	EU887875	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. araneiformis</i>	VEL	Velika Cave, Blato, Miljet, Croatia	EF217019	EF216944	EF216974.1	MK993720	EF216982	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. cassagnau</i>	TRI	Aghlia Triada Cave, Karistos, Eubea Island, Greece	EF217035	EF216931	EF216961	MK993737	EF216991	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. dalensi</i>	KEF	Kefalovrisi Cave, Argos, Argolide, Peloponnesus	EF217026	EF216929	EF216959	MK993745	EF216987	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. gasparoi</i>	CHI	Chirospilia Cave, Evghiros, Levkada, Ionian Islands	EF217008	EF216920	EF216950	MK993727	EF216976	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. giachinoi</i>	ORO	Megalospilio Cave, Monastirakion, Aitolio-Akarnania, Greece	EF217012	EF216922	EF216952	MK993731	EF216978	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. graeca</i>	PER	Perama Cave, Ioannina, Epiro, Greece	EF217013	EF216923	EF216953	MK993725	EF216979	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. hussoni</i>	IZB	Apano Skala Cave, Naoussa, Imathia, Greece	EF217031	EF216943	EF216973	MK993722	EF216990	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. insignis</i>	PAN	Panos Cave, Marathon, Athene, Attica, Greece	EF217054.1	EF216938	EF216968	MK993735	EF217000	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. ithakii</i>	ITA	Marmarospilia cave, Vathi, Ithaki Island, Ionian Islands	EF217006	EF216919	EF216949	MK993728	EF216975	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. kiriakii</i>	AGH	Kiriaki Cave, Korifè, Aghlia Kiriaki, Parga, Greece	EF217014	EF216924	EF216954	MK993732	EF216980	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. linderi</i>	SIR	Sirach Cave Eastern Pyrenees, France	AY793598	JF826039	AY793567	MK993708	JF826061	Allegrucci et al. 2019; Allegrucci et al. 2005
	<i>D. lustriae</i>	AND	Aghios Andreas Cave, Valtou M., Halkiopuli, Etolia, Greece	EU887901.1	EU887848	EU887863	MK993733	EU887878	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. makrykapa</i>	PKI	Paralia Kilidau Cave, Lamari, Eubea Island, Greece	EF217042.1	EF216941	EF216971	MK993739	EF216993	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. matsakisi</i>	ANA	Analipsi Cave, Pititsa, Achaia, Peloponnesus	EF217022	EF216927	EF216957	MK993744	EF216985	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. naxia</i>	ZEU	Zeus Cave, Filotas, Naxos Island, Cyclades, Greece	EU887909	EU887853	EU887868	MK993741	EU887882	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. paraskevi</i>	PAR	Aghlia Paraskevi Cave, Skotinon, Iraklio, Crete Island	EF217027	EF216942	EF216972.1	MK993747		Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. patrizii</i>	PET	Small cave, Petalas, Ionian Islands	EU887898	EU887847	EU887862	MK993729	EU887877	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. pavesii</i>	SPI	Drogarati Cave, Sami, Kefalonia Island, Ionian Islands	EF217010	EF216921	EF216951	MK993730	EF216977	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. petrochilosii</i>	JOA	Aghlia Joannis, Nea Pendeli, Athene, Attica, Greece	EF217053	EF216937	EF216967	MK993736	EF216999	Allegrucci et al. 2019; Allegrucci et al. 2009
	<i>D. remyi</i>	POZ	Pozarska Mala Pesterà, Loutrakio, Pella, Greece	AY793637	EF216939	EF216969	MK993721	EF217001	Allegrucci et al. 2019; Allegrucci et al. 2005

Table S1 (cont.).

Sub Family	Taxa	Code	Location	GenBank accession number					Reference
				12S	12S	16S	18S	28S	
Dolichopodainae	<i>D. sbordonii</i>	KAR	Karain Cave, Antalya, Turkey	EF217050	EF216936	EF216966	MK993748	EF216998	Allegrucci et al. 2019;
	<i>D. steriotisi</i>	ANT	Antropograva Cave, Klimatia, Kerkira, Corfu, Ionian Islands	EF217016	EF216925	EF216955	MK993726	EF216981	Allegrucci et al. 2009
	<i>D. thasosensis</i>	DRA	Drakotripa Cave, Panayia, Thasos Island, Kavala, Greece	EF217020	EF216926	EF216956	MK993724	EF216983	Allegrucci et al. 2019;
	<i>D. unicolor</i>	KAT	Kataphingi Cave, Selitsa, Messenia, Peloponnesus	EF217045	EF216940	EF216970	MK993746	EF216994	Allegrucci et al. 2009
	<i>D. vandeli</i>	HER	Hermes Cave, Orkomenos, Dhionisos, Beotia, Greece	EF217039	EF216932	EF216962	MK993734	EF216992	Allegrucci et al. 2019;
Rhaphidophorinae	<i>Rhaphidophora bicuspis</i>	MPN_CW5529	Bhutan	OR896623 (complete mt genome)					Allegrucci et al. 2009
	<i>R. quadrispina</i>		China	OL450400					This study
	<i>R. bilobata</i>	MPN_CW5545	Bhutan	OR896626 (complete mt genome)					Lu, X 2021 (retrieved from GenBank)
	<i>R. bhutanensis</i>	MPN_CW5483	Bhutan	OR896625 (complete mt genome)					This study
	<i>Stonychophora</i> sp.	MPN_ORT15	Bethel, Solomon Is.	OR896624 (complete mt genome)					This study
Troglophilinae	<i>Troglophilus adamovici</i>	A4E	Konya, Seydisehir, Tintazepe cave, Turkey	KY412419.1	KY412232	KY412279	KY412325	KY412372	Allegrucci et al. 2017
	<i>T. alanyaensis</i>	T22	Alanya, Dim Cave, Turkey	KY412414.1	KY412227	KY412274	KY412320	KY412367	Allegrucci et al. 2017
	<i>T. andreinii</i>	ANA	Putignano, Fico cave, Italy	KY412385.1	KY412198	KY412245	KY412292	KY412338	Allegrucci et al. 2017
	<i>T. bicakcii</i>	I1N	Konya, Derebucak, Bicacki cave, Turkey	KY412427.1	KY412240	KY412287	KY412333	KY412380	Allegrucci et al. 2017
	<i>T. brevicauda</i>	T9	Skrapar, Pirro cave, Albania	KY412398.1	KY412211	KY412256.1	KY412305	KY412351	Allegrucci et al. 2017
	<i>T. cavicola</i>	CAV	Bergamo, Grone, Belon cave, Italy	KY412388	KY412201.1	KY412248.1	MK993751	EF217002	Allegrucci et al. 2017
	<i>T. escaleraei</i>	A10	Karaman- Maras, Dongel cave, Turkey	KY412418.1	KY412231	KY412278	KY412324	KY412371	Allegrucci et al. 2017
	<i>T. ferzenensis</i>	ADA2B	Konya, Ferzene cave, Turkey	KY412422.1	KY412235	KY412282	KY412328	KY412375	Allegrucci et al. 2017
	<i>T. fethiyensis</i>	E1D	Mugla Fethiye, Guroluk cave	KY412424.1	KY412237	KY412284	KY412330	KY412377	Allegrucci et al. 2017
	<i>T. gajaci</i>	A9E	Icel Silifke, Cennet cave, Turkey	KY412421.1	KY412234	KY412281	KY412327	KY412374	Allegrucci et al. 2017

Table S1 (cont.).

Sub Family	Taxa	Code	Location	GenBank accession number					Reference
				12S	12S	16S	18S	28S	
Troglophilinae	<i>T. lagoi</i>	LAG	Rhodos Island, Greece	KY412407.1	KY412220	KY412267	KY412313	KY412360	Allegrucci et al. 2017
	<i>T. lazaropolensis</i>	T31	Macedonia, Lazaropole, Macedonia	KY412394.1	KY412207	KY412254	KY412301	KY412347	Allegrucci et al. 2017
	<i>T. marinae</i>	SAN	Santorini Island, Greece	KY412408.1	KY412221	KY412268	KY412314	KY412361	Allegrucci et al. 2017
	<i>T. neglectus</i>	NEG	Sagrado, Proteo cave, Italy	EU938374			KF570820	KF570948	Fenn et al. 2008; Allegrucci et al. 2017
	<i>T. ovuliformis</i>	T32	Zavala Vjetrenica, Herzegovina	KY412395	KY412208	KY412255	KY412302	KY412348	Allegrucci et al. 2017
	<i>T. ozeli</i>	E5D	Havran cave, Turkey	KY412426.1	KY412239	KY412286	KY412332	KY412379	Allegrucci et al. 2017
	<i>T. spinulosus</i>	SPI	Crete, 11.29 km northeast of Chania, near Gouverneto, Cave Spilaio Lera (Stavros), 184 m, Greece	KY412412.1	KY412225	KY412272	KY412318	KY412365	Allegrucci et al. 2017
	<i>T. tatyanae</i>	KAF	Artvin, Kafkasor, Turkey	KY412431.1	KY412244	KY412291	KY412337	KY412384	Allegrucci et al. 2017
	<i>T. zoiai</i>	MAY	Fokidos (Mt. Giona), Stromi, Mayer's Cave, Greece	KY412402.1	KY412216	KY412263	KY412310	KY412356	Allegrucci et al. 2017
Cyphoderrinae	<i>Cyphoderris monstrosa</i>			KM657332					Song et al. 2015
Prophalangopsinae	<i>Tarragoilus diuturnus</i>			NC_021397					Zhou et al. 2014

Note: *Diestrammena fengyangshanica* is described in student thesis which is publicly unavailable and also it is not included in the Orthoptera species list.

Table S2. The best-fitting partitioning scheme for phylogenetic analyses and divergence time estimation; 1) using 13 mitochondrial coding genes and 2) short sequences (117-taxa set).

	Partition method	BEST Substitution model	Subset partition	Alignment	Site model in BEAST	Non-standard substitution model implemented in BEAUti
1	13 Coding Genes	GTR+F+I+G4	atp6_co1_co2_co3_cytb_nd3	1-675 832-2373 2374-3042 3043-3828	GTR	Transition model, AC=GT, AT=CG and unequal base freq.
		GTR+F+I+G4	atp8_nd2_nd6	3829-4962 6901-7251	GTR	
		TIM+F+R4	nd1_nd4_nd4L_nd5	676-831 5872-6900 10615-11136 4963-5871 7252-8589 8590-8883 8884-10614	GTR	
2	Gene and codon position	GTR+F+R5	co1	1-1197;		
		TIM3e+I+G4	co1_pos1	1-1197		
		TN+F+R2	co1_pos2	2-1197		
		TPM2u+F+G4	co1_pos3	3-1197		
		GTR+F+I+I+R4	co1_16s	1198-1566 1567-2077		
		TIM2e+I+I+R2	co1_28s	2078-2628 2629-3314		

CHAPTER 3 | PHYLOGENY

Table S3. Mitochondrial genome annotation table of newly mapped sample sequences from North America, Bhutan, and Solomon Island. Asterix (*) represent incomplete TAA stop codon in the mRNA.

mt genome composition		CW5536 <i>Diestramima</i> sp1	CW5525 <i>Diestramima</i> <i>tsongkhapa</i>	CW5529 <i>Rhaphidophora</i> sp. 1	CW5483 <i>Rhaphidophorinae</i> gen. 2 sp. 1	CW5545 <i>Rhaphidophorinae</i> gen. 3 sp. 1	CW4347 <i>Ceuthophilus</i> sp.	ORT015 <i>Rhaphidophorinae</i> gen. 1 sp. 1	Direction
rRNA	12S rRNA	789	789	821	793	791	787	792	reverse
	16S rRNA	1359	1333	1364	1362	1359	1325	1239	reverse
	ATP6 CDS	678	678	678	678	678	678	678	forward
	ATP8 CDS	159	159	159	159	159	159	150	forward
	COX1 CDS	1545	1545	1545	1545	1545	1545	1545	forward
	COX2 CDS	691*	691*	690	690	690	688	688	forward
	COX3 CDS	789	789	789	789	789	789	789	forward
	CYTB CDS	1137	1137	1137	1137	1137	1137	1137	forward
CDS	ND1 CDS	951	951	951	951	951	951	951	reverse
	ND2 CDS	1029	1029	1029	1032	1029	1029	1029	forward
	ND3 CDS	354	354	354	354	354	354	354	forward
	ND4 CDS	1339*	1339*	1339*	1339*	1339*	1333*	1341	reverse
	ND4L CDS	294	294	294	294	294	294	294	reverse
	ND5 CDS	1737	1737	1732*	1732	1732*	1732*	1734	reverse
	ND6 CDS	528	528	528	528	528	519	528	forward
	Total coding genes length	11231	11231	11225	11228	11225	11208	11218	
tRNA	tRNA-Ala	63	63	63	64	63	65	64	forward
	tRNA-Arg	64	64	65	66	65	62	64	forward
	tRNA-Asn	67	67	67	67	67	64	66	forward
	tRNA-Asp	66	66	66	68	66	66	69	forward
	tRNA-Cys	66	66	71	66	67	66	60	reverse
	tRNA-Gln	69	69	69	74	69	67	69	reverse
	tRNA-Glu	68	68	68	69	68	64	66	forward

Table S3 (cont.)

mt genome composition		CW5536 <i>Diestramima</i> sp1	CW5525 <i>Diestramima</i> <i>tsongkhapa</i>	CW5529 <i>Rhaphidophora</i> sp. 1	CW5483 Rhaphidophorinae gen. 2 sp. 1	CW5545 Rhaphidophorinae gen. 3 sp. 1	CW4347 <i>Ceuthophilus</i> sp.	ORT015 Rhaphidophorinae gen. 1 sp. 1	Direction
tRNA	tRNA-Gly	67	67	66	67	67	61	67	forward
	tRNA-His	66	66	63	65	64	62	68	reverse
	tRNA-Ile	65	65	66	66	66	65	70	forward
	tRNA-Leu	67	67	66	67	67	68	66	forward
	tRNA-Leu	65	65	65	67	66	65	64	reverse
	tRNA-Lys	70	69	69	70	69	69	71	forward
	tRNA-Met	69	69	69	71	70	68	69	forward
	tRNA-Phe	66	66	66	66	67	63	66	reverse
	tRNA-Pro	67	67	66	66	66	68	69	reverse
	tRNA-Ser	70	70	69	70	69	68	71	forward
	tRNA-Ser	68	68	67	67	67	66	70	forward
	tRNA-Thr	64	64	67	69	70	67	68	forward
	tRNA-Trp	68	68	69	66	68	68	71	forward
	tRNA-Tyr	68	68	66	66	67	66	50	reverse
	tRNA-Val	71	71	72	70	71	71	71	reverse
Total genome length		14853	14826	14885	14870	14854	14769	14718	

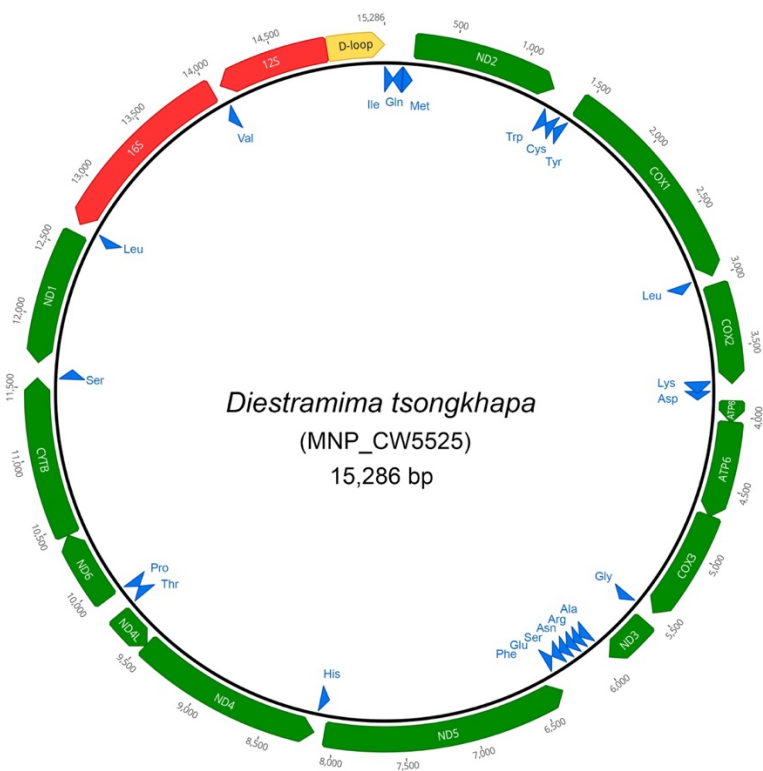


Figure S1. The mitochondrial genome of *Diestramima tsongkhapa* (MPN_CW5525) with 13 protein coding genes in green, 2 rRNAs in red, 22 tRNA genes in blue and A + T rich repeat region in yellow. The direction of each annotation is indicated with arrow.

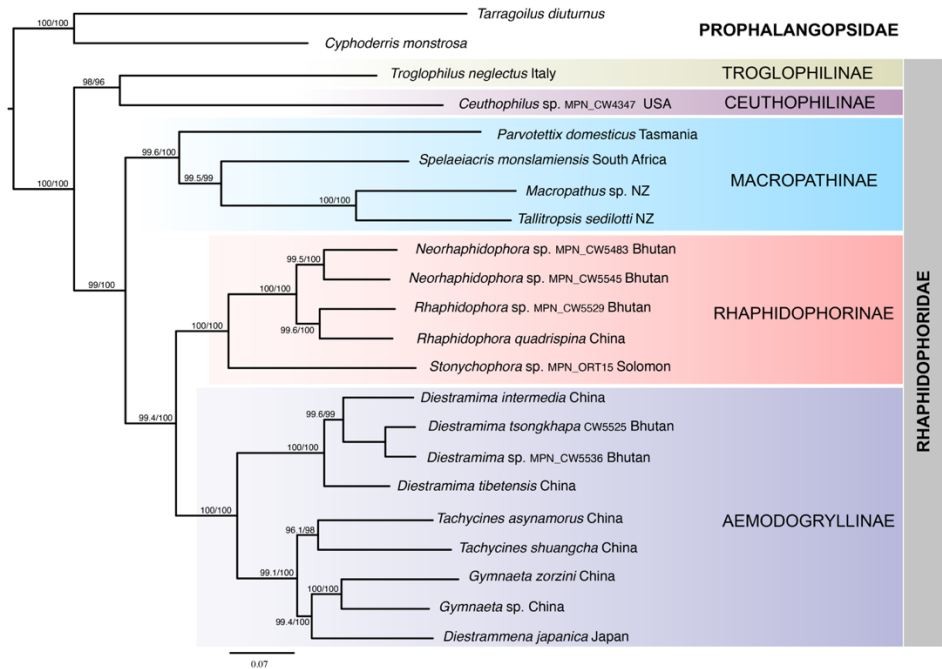


Figure S2. Evolutionary relationships of Rhaphidophoridae inferred from nucleotide alignment of 13 concatenated mitochondrial protein coding genes (11,124 bp) by ML analysis. Values on nodes are Sh-aLRT/bootstrp support values (1000 replicates).

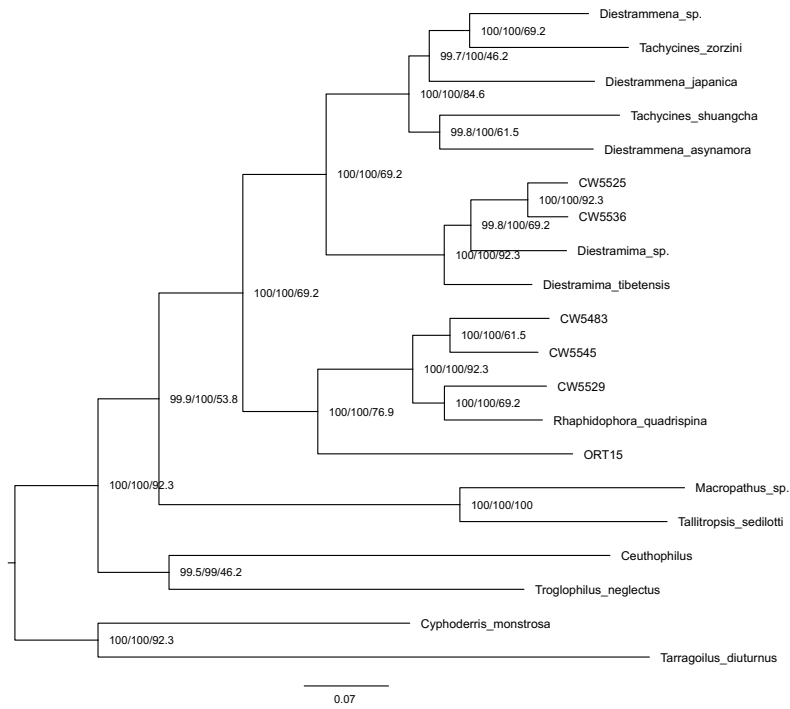


Figure S3. ML tree generated using IQ TREE. Gene concordance factor of complete mitochondrial coding genes of Rhaphidophoridae. The values on the nodes represent ultra-fast bootstrap values and SH-aLRT values of reference tree (Gene tree) and gene concordance factor.

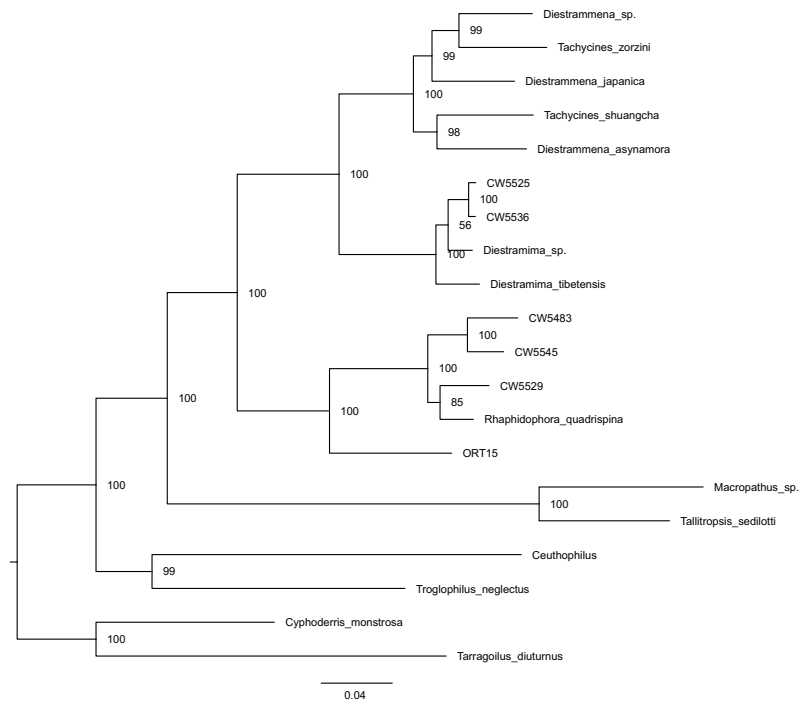


Figure S4. ML tree generated using IQ TREE. Phylogenetic tree constructed using the complete mitochondrial amino acid alignment sequence of Rhaphidophoridae. The values on nodes represent Ultra-fast bootstrap values.

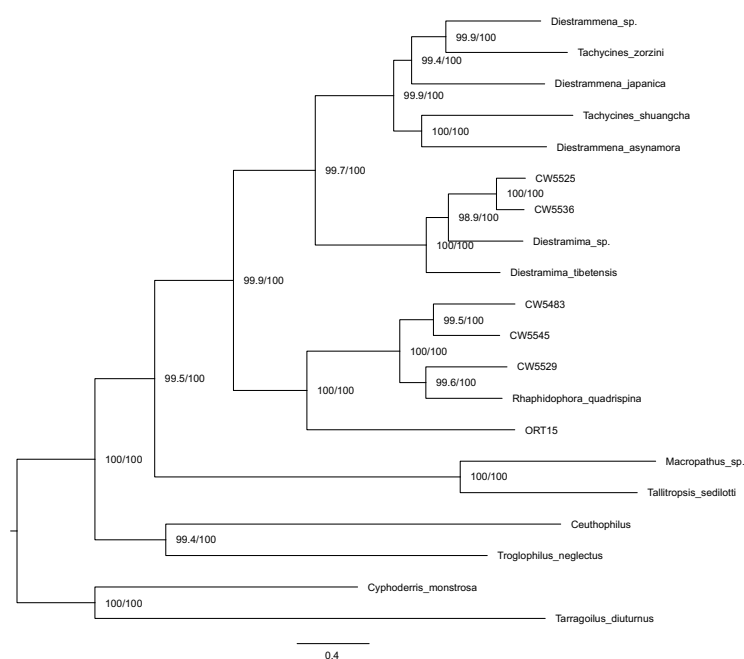


Figure S5. ML tree generated using IQ TREE. Complete mitochondrial DNA phylogeny of Rhaphidophoridae infer on 13 coding genes partition by gene and codon positions (52 partitions). The values on the nodes represent Ultra-fast bootstrap and SH-aLRT values.

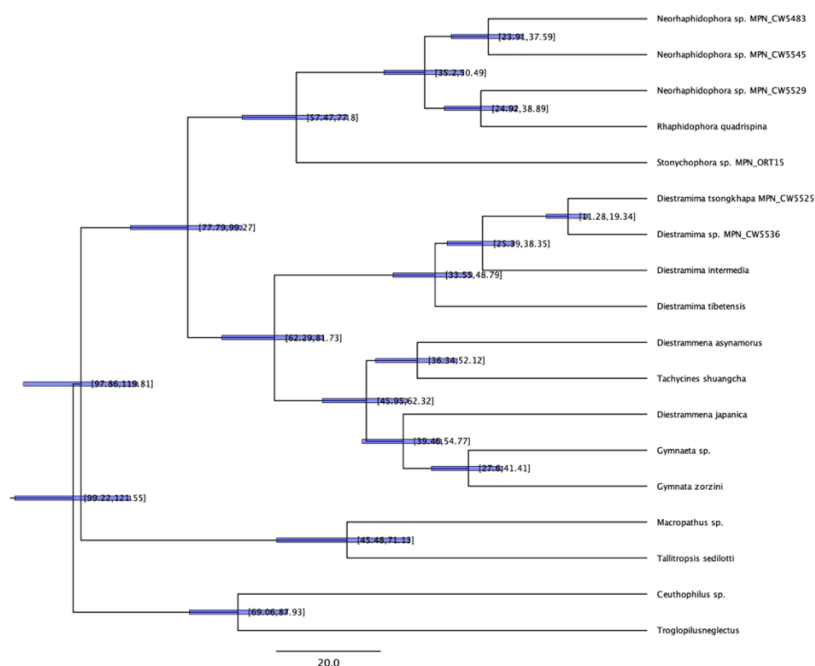


Figure S6. Chronogram of Raphidophoridae emphasising Asian lineages inferred by Bayesian analysis using points corresponding to divergent of Troglophilinae from Ceuthophilinae and Raphidophorinae and Aemodogryllinae from Macropathinae from previous analysis based on short sequences and secondary landscape-calibration (Allegrucci & Sbordoni, 2019). Node bars represent 95% Highest Posterior Density credibility intervals

CHAPTER FOUR

Spatial and genetic patterns in cave associated camel crickets (Rhaphidophoridae): insights from caves of Maniapoto karst, New Zealand and Okinawa, Japan.

ABSTRACT

Caves represent islands of habitat for many organisms that are morphologically and physiologically adapted to living underground. Differences in geological connectivity among cave systems and the extent to which species are cave dependent are expected to affect their dispersal ability, biogeographical isolation, and spatial genetic structure. In this study, we sampled two coexisting troglophilic camel crickets (Rhaphidophoridae): *Pachyrhamma waitomoense* and *Pallidoplectron turneri* from Maniapoto karst, New Zealand and *Neotachycines obliquofasciatus* from limestone caves of Okinawa Island, Japan. We sequenced 806–1339 bp of DNA from the mitochondrial gene cytochrome oxidase I (COI) for a total of 140 individuals of these newly sampled taxa. We assess the degree of connectivity across a small geographic scale to determine whether the cave samples carry genetic signal that suggest they function as discrete populations. We then compare spatial structure at the same locus within raphidophorid species from around the world. In sampled species we observed that genetic structure was concordant with isolation by distance in six of nine species. No evidence of isolation by distance was found among forest populations of *Pristoceuthophilus celatus* and cave inhabiting *Ceuthophilus* (*Ceuthophilus*) sp. from Northern America. Within Maniapoto karst species, gene flow is not entirely absent, with rare sharing of haplotypes among cave systems detected. Most of the genetic diversity within *Pachyrhamma waitomoense* and *Pallidoplectron turneri* was partitioned among cave samples and cave systems, suggesting successful movement between caves is not frequent. In contrast, no evidence of ongoing gene flow was detected among the Okinawa cave populations of *Neotachycines obliquofasciatus*, with significant genetic differentiation observed between populations. Although their high level of genetic differentiation among caves suggests that our sample taxa are primarily cave dwellers, we detected a signal of isolation by distance implying gene flow. Lack of genetic sharing among *N. obliquofasciatus* cave populations and their presence outside caves may be a recent outcome of changing environments.

KEY WORDS

Waitomo caves, Okinawa, troglophile, troglomenes, gene flow, genetic structure, mitochondrial DNA, phylogeography, *Pachyrhama waitomoense*, *Pallidoplectron turneri*, *Neotachycines obliquofasciatus*.

INTRODUCTION

Many animals use caves for all or some of their lives. Species that specialise in subterranean ecosystems are characterised by a set of morphological and physiological traits (collectively referred to as troglomorphy) such as reduced sight, reduced pigmentation, long appendages, and slow metabolic rates (Christiansen, 2012). But many animal species that use cave environments also live above ground, with adaptations to dark humid stable environments that can also be found in forests. Animals that use caves differ in how much of their lives are spent underground and their degree of adaptation to cave environments, resulting in the terms troglophiles (species that thrive in caves) and troglonexes that use caves for part of their lifecycle but have no special adaptation for cave ecosystems (Hubbell, 1936; Leroy, 1967; Richards, 1968). The degree to which cave-dwelling species can exist outside caves influences the opportunity of mixing among populations and thus, spatial population genetic structure (Caccone, 1985; Guzik et al., 2011; Pavlek et al., 2022). Species strictly associated with caves might show population dynamics similar to those found among populations on separate islands with strong population differentiation. In contrast, species that are not cave obligates are likely to exhibit isolation by distance due to gene flow among adjacent populations.

Contrasting the genetic structure of related taxa with a range of degrees of dependency on cave habitats helps identify population cohesion (Caccone, 1985). Orthoptera of the family Rhaphidophoridae (camel crickets) offer an excellent opportunity to contrast the degree to which cave systems isolate populations (Table 1). The Rhaphidophoridae have a global distribution across the Holarctic, Afrotropical, Neotropical, Australian, Oriental, and temperate Palearctic regions, encompassing 922 recognised species in nine subfamilies and a wide range of landscapes (Allegrucci et al., 2009; Dorji et al., 2025; Allegrucci et al., 2005).

According to Di Russo et al., 1994 and Martinsen et al., 2009, more than half of the 300 camel cricket species at that time were considered to be cave dwellers with body shapes considered appropriate for living in caverns: elongated legs and antennae, reduced eyes, and little pigmentation. Troglomorphy in invertebrates often involves loss of pigments and functional eyes (Christiansen, 2012). Complete lack of colour of teguments is uncommon, although species of Dolichopodinae and Ceuthophilinae are typically paler than Macropathinae and Aemodogryllinae (Di Russo & Sbordon, 1998). Only two species display troglomorphic extremes, including lack of eyes. The eyeless (anophthalmic) species of camel crickets are *Diestrammena caeca*, discovered in a cave in Assam, India, and *Eutachycines cassani*,

described from Grotte Marie Cassan, Laos (Chopard, 1924, 1954; Rampini et al., 2013; Richards, 1968). In addition to these, several species of *Tachycines* (Aemodogryllinae) have reduced eyes, e.g., *Tachycines shibenzhangii* from Southeast and East Asia (Lin et al., 2023; Zhou & Yang, 2022). However, most *Tachycines* species are classified as troglophiles or troglonexes, inhabiting cave entrances and humid forest environments, suggesting broader habitat preference in the group (Jiao et al., 2008; Zhang & Liu, 2009). Even in tropical wet forest, a large proportion of camel crickets are considered cave-dwelling (Gunn, 2004; Inchoedchay et al., 2025; Zhou & Yang, 2022), although the closeness of this association is not well-studied.

Troglophilic species are mostly forest dwellers occurring in tropical moist and humid areas and in epigeal environments (Table 1). Many cave crickets roost during the daytime in caves but can also be found in humid microhabitats such as rock crevices, under tree bark, leaf litter, and hollow logs (Carchini et al., 1991; Howarth, 1993; Lavoie et al., 2007; Richards, 1954; Allegrucci et al., 2005). Within Carlsbad caverns (USA), a spectrum of troglomorphic adaptation is apparent. *Ceuthophilus longipes* (trogllobites) is considered to be the most cave-adapted, living deep within cave systems (Hubbell, 1936; Lavoie et al., 2007). Compared to related species, *Ceuthophilus longipes* is paler and has longer, more slender femora, antennae and body and low metabolic rate (Northup et al., 1993; Northup & Crawford, 1992). Congenerics such as *Ceuthophilus carlsbadensis* and *Ceuthophilus conicaudus*, and the sister genus *Pristoceuthophilus*, can be found foraging near cave entrances and occasionally in forest surface under logs and stones (Elliott et al., 2017; Lavoie et al., 2007).

In New Zealand species of *Macropathus* can complete their life cycle in caves but are also capable of dispersing between cave systems, with solitary individuals observed outside cave areas (Richards, 1968). Even within the predominantly cave-associated genus *Dolichopoda* in Italy, several populations thrive in rock crevices and moist woodland ravines, with individuals sometimes observed outside caves at night, and some have also colonised human-made hypogean environments (Baccetti & Capra, 1959; Sbordoni et al., 1987, 1991). The similarity in morphology and behaviour among Rhaphidophoridae species suggests overlapping tolerance to similar micro-habitats and similar dispersal capabilities between caves systems (Di Russo et al., 1994). The conserved ecological niche of camel crickets suggests most species possess the potential to cross subterranean and forest environments where light levels are low and humidity is high.

As caves often function as isolated habitat patches, their animal populations resemble those of island systems. However, variation in ability to dispersal between caves and the duration of cave occupation contribute to differing degrees of population differentiation (e.g., *Ceuthophilus gracilipes* – Cockley et al., 1977; *Dolichopoda* – Cesaroni et al., 1997). Mark-recapture has revealed short-range dispersal of individuals in artificial caves in Rome although this is less apparent in the same species occupying natural caves (Rampini et al., 1983). Population structure of two lineages of co-occurring cave dwelling camel crickets in Texas revealed geographic structure and contrasting levels of mtDNA diversity that suggest different timing of colonization, population size and/or movement among caves (Weckstein et al., 2016). In general, forest-dwelling populations of camel crickets are expected to be more interconnected and genetically similar to each other compared to cave restricted lineages (Caccone, 1985; Caccone & Powell, 1987). Thus, the degree to which a lineage is adapted to cave environments should be considered when examining patterns of genetic diversity within cave-dwelling Rhabdophoridae species, as varying levels of adaptation may influence genetic structure and variability (Sbordoni et al., 1991).

I generated new data for phylogeographic analysis to compare the scale and pattern of genetic diversity of three species: two co-occurring troglomorphic species, *Pachyrhamma waitomoense* and *Pallidoplectron turneri* in the Maniapoto Karst, New Zealand, and troglomorphic *Neotachycines obliquofasciatus* from Okinawa Island, Japan. These data were used to assess the degree of connectivity among populations samples at small geographic scales to test whether cave samples carry genetic signal indicating they function as discrete populations. I then compared spatial structure at the same gene locus among cave-dwelling Rhabdophoridae around the world utilising available genetic data. My hypothesis is that the greater the difference between cave and surface conditions, and the more ecologically specialised the species, the stronger the genetic signal would be for population partitioning in the cave habitats. Compared to troglophiles or troglomenes (epigean), troglobites might be expected to generally exhibit lower gene flow among cave populations (Sbordoni et al., 2000). In regions characterised by isolated cave systems such as in karst landscapes, populations of cavernicolous (cave dwellers) species are expected to experience restricted dispersal and gene flow, leading to pronounced genetic differentiation and well-resolved phylogeographic structure. In contrast, forest dwelling relatives inhabit more continuous habitat and therefore are expected to show reduced genetic structuring and lower levels of differentiation (Allegrucci et al., 1997). The reliance of troglobitic species on subterranean environments constrain their dispersal opportunities, as

these organisms are often unable to survive or disperse through surface environments. Consequently, populations can be isolated to individual caves or subterranean networks, leading to deeper genetic divergence (Sbordoni et al., 2000). The rate of gene flow among cavernicolous populations can be predicted from cave geology, its surface vegetation profile, and the geographic distance separating populations (Caccone, 1985).

MATERIALS AND METHODS

New Sampling

Maniapoto karst area in west-central North Island is a landscape characterised by Oligocene–Miocene limestone extending about 1000 km², underground rivers and sinkholes developed by high rainfall, dense forest, and erosion, resulting in diverse subterranean habitats (Molloy & Smith, 2002; Williams, 1992). Central to this karst are the well-known Waitomo caves, one of the best-studied karst systems in the world (Ford & Williams, 1989; Gillieson, 1996). Both *Pachyrhamma waitomoense* and *Pallidoplectron turneri* appear to be endemic to the Maniapoto karst, with no documented presence beyond these caves (Cook et al., 2010; Richards, 1958). However, other species of *Pachyrhamma* have wider ranges in New Zealand and sometimes occur in caves and thus raising the question of whether the cave populations of *P. waitomoense* and *P. turneri* represent isolated specialists or are each part of broader, continuous distributions.

Sampling of *P. waitomoense* Richards, 1958 and *P. turneri* Richards, 1958 was undertaken at 17 caves in the Maniapoto karst region of Waikato, New Zealand (Fig. 1 and Table 2). The Waitomo Caves, central to this karst system, are globally recognized for their rich invertebrate fauna, including Opiliones, *Arachnocampa* glow-worms, beetles, and cave wētā (Ford & Williams, 1989; Gillieson, 1996). Prior to human arrival about 750 years ago (Matisoo-Smith, 2017), this area would have been covered in continuous forest, now fragmented by pasture interspersed with roads, streams, and townships. However, there are no major natural geographic barriers such as mountain ranges or large rivers. Hitherto these Rhaphidophoridae species have only been recorded in caves. Between one and six caves were sampled at each of five areas spanning about ~47 km apart (Table 3). Within each area cave entrances are within ~25 m (between Charlie’s Crack and Aranui) to ~2 km (Kuratahi and Thunder fall) of each other.

Table 1. Examples of cave-associated Raphidophoridae and their traits. The remark “No” means no record of species outside cave system based on available literature.

Ecological status	Species	Country	Cave association	Outside dispersal	Reference
Troglobite	<i>Diestrammena caeca</i>	Assam, India.	Obligatory to cave system. Anophthalmic.	No	Chopard, 1924
Troglobite	<i>Eutachycines cassani</i>	Grotte Marie Cassan, Laos.	Obligatory to cave system. Anophthalmic.	No	Chopard, 1954; Rampini et al., 2013
Troglobite	<i>Diestrammena G. caverna</i>	Hubie, China.	Found in cave.	No	Jiao et al., 2008
Troglobite	<i>Tachycines G. zhongi</i>	Daxiao Dong, China.	Reduced eye. Found within cave in weak light and dark zones.	No	Zhou & Yang, 2022
Troglobite	<i>Tachycines G. shibenzhangii</i>	Xuehua Cave, China.	Ommateum extremely degenerated. Found in cave.	No	Zhou & Yang, 2022
Troglophile	<i>Micropathus cavernicolus</i>	Tasmania.	Complete its life cycle in caves but comes out for forage during night.	Yes	Richards, 1968; Beasley-Hall & Eberhard, 2025
Troglophile or Troglaxene	<i>Micropathus tasmaniensis</i>	Tasmania.	Complete its life cycle in caves but comes out for forage during night.	Yes	Richards, 1968; Hamilton-Smith, 1967; Beasley-Hall & Eberhard, 2025
Troglophile	<i>Ceuthophilus secretus</i>	Edwards Plateau, Texas, US.	Forage outside during day but complete life cycle in cave.	Yes	Taylor et al., 2005
Troglophile	<i>Ceuthophilus longipes</i>	Carlsbad Caverns, US.	Living deep in cave.	Yes	Hubbell, 1936; Lavoie et al., 2007
Troglophile	<i>Pallidoplectron turneri</i>	Waitomo Caves, New Zealand.	Complete life cycle in cave.	Yes	Richards, 1961b
Troglophile	<i>Pachyrhamma waitomoense</i>	Waitomo Caves, New Zealand.	Complete life cycle in cave but also found outside.	Yes	Richards, 1961b
Troglophile	<i>Neotachycines furukawai</i>	Honshu, Shikoku, Okinawa, Japan.	Prefers wet forest floors and caves.	Yes	Sugimoto & Ichikawa, 2003
Troglophile	<i>Neotachycines obliquofasciatus</i>	Okinawa, Iheya and Hamahiga Islands, Okinawa, Japan.	Found in cave entrance and wet forest and along streams.		Sugimoto & Ichikawa, 2003
Habitual troglaxenes or troglaphile	<i>Ceuthophilus conicaudus</i>	Carlsbad Caverns, US.	Leave the cave only to forage, and all other aspects of their life cycle occur in caves.	Yes	Lavoie et al., 2007
Habitual troglaxenes or troglaphile	<i>Hadenoeus subterraneus</i>	Mammoth cave, Kentucky, US.	Leave the cave only to forage, and all other aspects of their life cycle occur in caves.	Yes	Hubbell & Norton, 1978; Lavoie et al., 2007
Troglaxene	<i>Ceuthophilus carlsbadensis</i>	Carlsbad Caverns, US.	Found in cave entrance near bat guano.	Yes	Lavoie et al., 2007
Troglaxene	<i>Ceuthophilus stygius</i>	Kentucky, USA.	Use caves as refuge and complete life cycle in forest.	Yes	Lavoie et al., 2007
Troglaxene	<i>Ceuthophilus gracilipes</i>	Eastern US.	Found in caves and in the forest under logs and loose bark.	Yes	Cockley et al., 1977

All insects were collected at cave entrances during November 2006 with permission from landowners, and support from New Zealand Department of Conservation staff. The numbers of specimens taken from each cave varied depending on their apparent abundance in the cave, ease of capture and time limitations (Table 3). Sampling was limited to juveniles and identification followed Richards (1958).

Okinawa Island is the largest of the Ryukyu Islands, which originated from a continental margin arc into an island arc due to back-arc spreading in the Okinawa Trough that ultimately led to the islands' subsidence and isolation (Osozawa et al., 2012). The island coastline is bordered by coral reefs and raised coral limestone with Palaeozoic strata in the northern region and Tertiary deposits in the south (Fujiki & Ozawa, 2008). Okinawa and surrounding islands have abundant Quaternary cave systems and tropical forests which support unique and endemic terrestrial fauna from both modern and late Pleistocene periods (Watanabe et al., 2023). More than 4500m of cave passage has been surveyed, with an underground stream, bat colony and subterranean invertebrate communities (Gillieson, 2003). Among these, Gyokusendo Cave is one of the largest in the southern part of the island, extending over 4 km and estimated to have formed in the early Pleistocene within Ryukyu limestone (Gillieson, 2003).

Neotachycines obliquofasciatus endemic to Okinawa and surrounding islands is primarily found in natural limestone caves, with some populations inhabiting copper mine tunnels, and occasionally few individuals observed outside their caves in moist forests and along streams (Sugimoto & Ichikawa, 2003). Despite the relatively small geographic area, *N. obliquofasciatus* populations remain highly isolated due to topology of islands, which includes low mountains, secondary forest, agricultural fields (cane and pineapple fields), settlements, and streams and rivers. For this study, *N. obliquofasciatus* specimens were collected in 2024 within limestone caves and copper mines across Okinawa Prefecture, including Iheya, a small northern island (Fig. 1). Cave samples were spread across area spanning 60 km² with a minimum distance of ~12 km (between Onno and Ginoza) and a maximum distance range of ~101 km (between Iheya and Tomigusu) between each sampled cave. Two to seven individuals were sampled from each cave system (Tables 2, 3), and all the specimens were identified by Mr. Masashi Sugimoto, the original describer of the species.

Additional Data

Additional genetic data were harvested for suitably sampled Rhaphidophoridae reported from a range of geographical and ecological settings in North America, Europe and New Zealand (Allegrucci et al., 2014; Weckstein et al., 2016; Allegrucci et al., 2005) (Table 2). This allowed inclusion of *Dolichopoda azami* found in caves and cave entrances across the Maritime Alps in Northwest Italy, *Ceuthophilus* (subgenera *Geotettix* and *Ceuthophilus*), which inhabit the Central Edwards Plateau karst in Texas, two *Pristoceuthophilus* species (*P. celatus* & *P. cercalis*) from British Columbia and southern Alaska. These latter species are not restricted to cave habitats and can also be found in forests, grasslands, and occasionally in open fields. The widespread New Zealand forest species, *Talitropsis sedilloti* was included (Table 2 and Fig. 1).

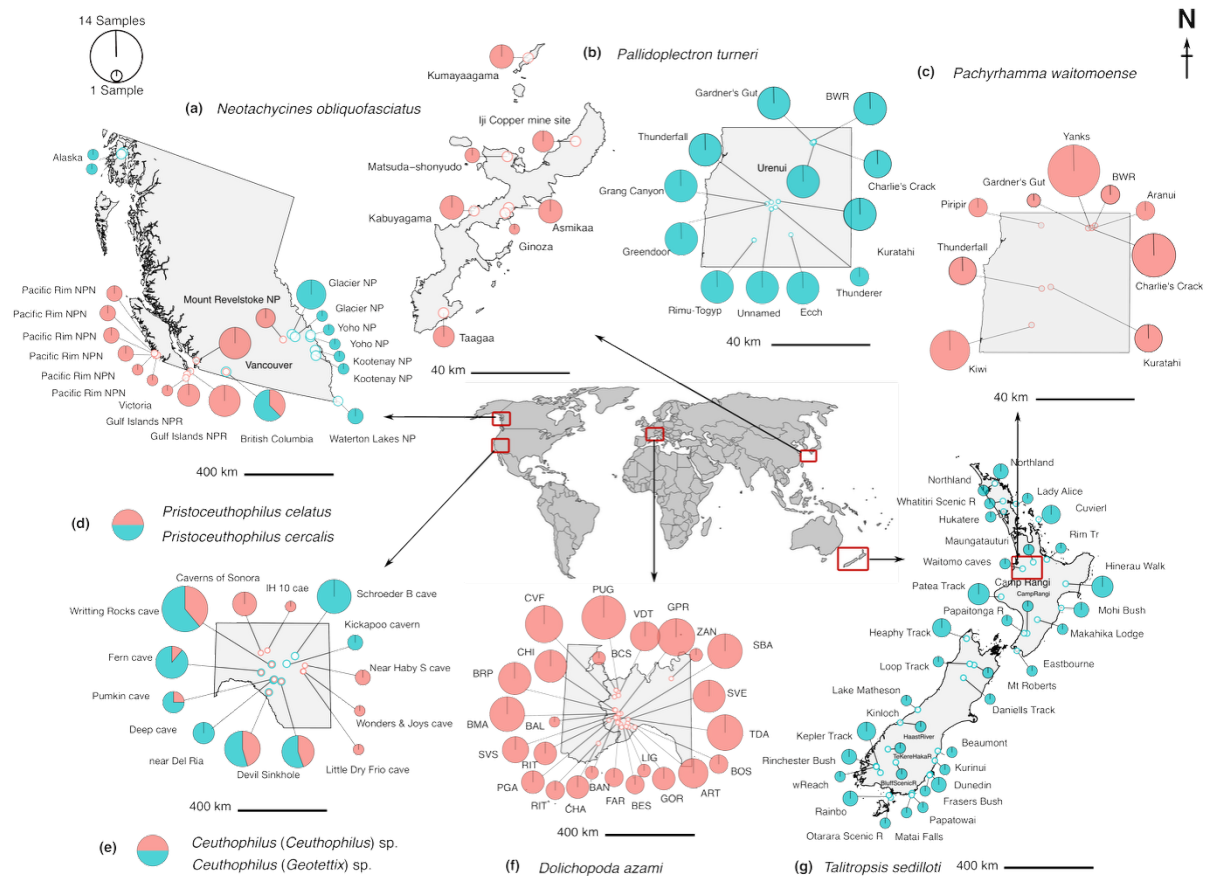


Figure 1. Global location of Rhaphidophoridae taxa examined with details of the number of individuals sampled from different regions for this study. Localities and sampled individuals of the species *Ceuthophilus* and *Pristoceuthophilus* and *Dolichopoda azami* and *Talitropsis sedilloti* are based on published data presented in table 2. The size of each circle indicates the total number of individuals, while the pie chart illustrates the proportion of individuals for each species from the sampled locality.

DNA Extractions, Amplification and Sequencing

Mitochondrial DNA sequences were generated for newly sampled cave-dwelling rhabdophorids. DNA was extracted from leg muscle using a ‘salting-out’ approach (Sunnucks & Hales, 1996) for *Pachyrhamma waitomoense* and *Pallidoplectron turneri* (Trewick & Morgan-Richards, 2005) and Quantabio Extrat kit for *Neotachycines* species. Mitochondrial Cytochrome Oxidase I (COI) gene was amplified using polymerase chain reaction (PCR) a combination of primers: L2-N-3014, C1-J-2195 (Simon et al., 1994), mtd12_wetaR (Bulgarella et al., 2014), LCO1490 and HCO2198 (Folmer et al., 1994). Amplifications were performed in 10 µL reactions containing 1.0 µL of 10x PCR buffer, 0.8 µL of 25 mM MgCl₂, 0.2 µL of each 2 mM dNTP, 0.4 µL of each 10 µM primer, 0.08 µL of ABGene Taq DNA or DreamTaq DNA polymerase. Thermal cycling involved 2 minutes of initial denaturation at 94°C then 38 cycles of 94°C for 15 s, 52°C for 15 s and 72°C for 90 s. PCR products sequenced using BigDye™ Terminator chemistry (Perkin-Elmer Applied Biosystems) with AB13730 genetic analyser (Applied Biosystems Inc.). DNA sequences were aligned and checked for insertion/deletions, amino acid translation and homology using Geneious v9 (Kearse et al., 2012).

Table 2. Raphidophorid species included in new population genetic analyses. Species highlighted in grey were excluded from phylogeography analysis due to sample sizes < 21.

	Species	Locality and Country	Habitat	No. of location	Total sample	COI position	Seq. Length (bp)	Reference
1	<i>Neotachycines obliquofasciatus</i>	Okinawa Island, Japan	Cave	6	26	100-727 & 841-1552	1339	This study
2	<i>Talitropsis sedilloti</i>	North and South Island, NZ	Forest	35	52	398-1142	744	Goldberg & Trewick, 2011; Trewick, 2000
3	<i>Talitropsis crassicuris</i>	Chatham Island, NZ	Open field/bush	9	20	372-1173	801	Trewick, 2000
4	<i>Talitropsis megatibia</i>	Chatham Island, NZ	Open field/bush	5	12	372-1186	814	Goldberg & Trewick, 2011
5	<i>Pallidoplectron turneri</i>	Maniapoto Karst, North Island, NZ	Cave	9	48	295-1101	806	This study
6	<i>Pachyrhamma waitomoense</i>	Maniapoto Karst, North Island, NZ	Cave	12	66	295-1101	806	This study
7	<i>Ceuthophilus</i> (<i>Ceuthophilus</i>) sp.	Central Edwards Plateau, Texas, USA	Cave	10	28	264-1114	850	Weckstein et al., 2016
8	<i>Ceuthophilus</i> (<i>Geotettix</i>) sp.	Central Edwards Plateau, Texas, USA	Cave	8	49	264-1114	850	Weckstein et al., 2016
9	<i>Dolichopoda azami</i>	Maritime alps, Italy	Cave	25	104	48-1457	951	Allegrucci et al., 2014
10	<i>Dolichopoda geniculata</i>	Italy	Cave and cave entrance	10	14	297-1254	957	Allegrucci et al., 2005
11	<i>Pristoceuthophilus cercalis</i>	British Columbia, Canada and Alaska, USA	Forest	10	21	115-608	493	deWaard et al., 2019
12	<i>Pristoceuthophilus celatus</i>	British Columbia, Canada	Forest	12	37	94-605	513	deWaard et al., 2019

Population Genetics and Phylogeography

Genetic diversity within populations of nine camel cricket species were inferred using DnaSP v6.12 (Librado & Rozas, 2009). Haplotype (h) and nucleotide (π) diversity indices (Nei, 1987) were computed separately for each taxon and population sample (supplementary table 1). Using NEXUS haplotype data from DnaSP, median-joining haplotype networks (Bandelt et al., 1999) were produced using the statistical parsimony network algorithm implemented in PopArt (Leigh & Bryant, 2015). All figures were edited in Inkscape v2 to enhance visualisation. ARLEQUIN v3.5 (Excoffier et al., 2005) was used to analyse molecular variance (AMOVA), with the statistical significance of variance components tested with 1023 permutations, and results with p -values < 0.05 were considered statistically significant. Two alternative groupings for *P. waitomoense* and *P. turneri* were tested: (1) all caves as one group, and (2) caves grouped by their relative geographic position to five areas.

Mismatch distributions of pairwise mutational differences among individuals (Rogers & Harpending, 1992), were used to explore the demographic among newly sequenced populations using DNASP (Rozas et al., 2017). A model of spatial expansion was used for comparison, as stable populations typically have multimodal mismatch distributions, whereas populations that have experienced recent demographic expansion generally display unimodal distributions (Harpending, 1994; Rogers & Harpending, 1992). For example, multimodal distributions in New Zealand tree wētā have been interpreted as evidence of large population sizes (Morgan-Richards et al., 2017; Slatkin & Hudson, 1991). Goodness of fit to a unimodal mismatch distribution was tested using Harpending's raggedness index (Harpending et al., 1993) and the sum of squares deviation (SSD; Schneider & Excoffier, 1999) using ARLEQUIN v3.5 with 1,000 bootstrap resamples. A non-significant result indicates a good fit to the unimodal model, supporting the hypothesis of population expansion.

To investigate spatial partitioning, I calculated pairwise Φ_{ST} (ϕ_{iST}) and average haplotype genetic distance for species samples in each cave system using the *pegas* and *ape* R packages (Paradis, 2010; Paradis et al., 2004). I tested for a signature of isolation by distance (IBD) by applying the Mantel test (Diniz-Filho et al., 2013; Mantel, 1967; Tamura & Nei, 1993) to compare pairwise population (Φ_{ST}) (or mean haplotype genetic distance) with geographic distance estimated based on the shortest distance between two points on an ellipsoid, using 1000 bootstrap replicates. If the distribution of camel crickets is primarily influenced by the spatial arrangement of caves, the resulting limited gene flow among cave populations is not expected

to result in a significant pattern of IBD. However, caves might represent convenient sampling aggregations of species that also occur (now or in the recent past) throughout the intervening habitat matrix, in which case a stronger correlation between geographic and genetic distance (IBD) would be expected.

RESULTS

Amplification products and COI mtDNA sequences were obtained from 146 individuals consisting of a 1339 bp alignment of *Neotachycines obliquofasciatus* and 806 bp for both *Pachyrhamma waitomoense* and *Pallidoplectron turneri*. To simplify comparison of the newly sequenced taxa, statistics were calculated after aligning data for *P. turneri*, *P. waitomoense* and *N. obliquofasciatus* and selecting only the homologous sites present in all three species. The resulting alignment comprised 693 nucleotides of homologous sequence. Among 48 *P. waitomoense* sequences there were 11 variable nucleotide positions among 693 bp (~1.58%) that resulted in 11 unique haplotypes (*pw1*–*pw11*), while 66 *P. turneri* homologous sequences there were 27 variable nucleotide positions (~3.89 %) and 12 unique haplotypes (*pt1*–*pt12*). In keeping with these different levels of haplotype variation, genetic diversity statistics for these two New Zealand species were quite distinct; *P. turneri* showed higher haplotype nucleotide diversity per population sample than *P. waitomoense* sampled from the same landscape (Table 2). Maximum Jukes Cantor genetic distances within the sample were 0.0339 for *P. turneri* and 0.0072 for *P. waitomoense*. The homologous sequences of 693 bp COI from 26 individuals of *N. obliquofasciatus* from Okinawa had higher genetic diversity compared to *P. waitomoense* and *P. turneri*, with 64 variable nucleotide positions (~9.24 %), 18 unique haplotypes, 0.954 haplotype diversity, and 0.0507 (~5.07%) maximum Jukes Cantor genetic distance.

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Table 3. Sampling statistics and genetic characteristics of haplotype diversity among population samples. Abbreviations used in the table are as following: Number of individuals (n), number of Segregating sites (S), number of haplotypes (h), Haplotype diversity (Hd), nucleotide diversity (π) (Nei, 1987). Statistics were calculated using DnaSP (Librado & Rozas, 2009).

(a) Molecular variation among mtDNA COI (693 bp) of 48 *Pachyrhamma waitomoense* in nine Maniapoto cave population samples, New Zealand.

Area	Cave	n	S	h	Hd	n	Haplotypes (pw1-pw11)										
							1	2	3	4	5	6	7	8	9	10	11
Piripiri	Piripiri	2	0	1	0	0									2		
Waitomo	All	29	9	9	0.744	0.00219											
	BWR1	2	1	2	1	0.00144	1		1								
	Charlie's Crack	10	5	5	0.822	0.00212	4			2	2	1	1				
	Gardner's Gut	1	0	1	0	0								1			
	Aranui	2	4	2	1	0.00577	1	1									
Puketiti	Yanks	14	5	5	0.659	0.00184	8			3			1	1			1
	All	8	3	2	0.250	0.00108											
	Kuratahi	4	3	2	0.5	0.00216	1								3		
	Thunderfall	4	0	1	0	0									4		
Mahoenui	Kiwi	9	1	2	0.389	0.00056									7	2	
All		48	11	11	0.788	0.00303	14	1	1	5	2	1	2	2	14	2	1

(b) Molecular variation among mtDNA COI (693 bp) of 66 *Pallidoplectron turneri* in 12 Maniapoto cave population samples, New Zealand.

Area	Cave	n	S	h	Hd	n	Haplotypes (pt1-pt112)											
							1	2	3	4	5	6	7	8	9	10	11	12
Waitomo	All	21	23	5	0.736	0.01452												
	BWR1	6	23	4	0.800	0.01664	1	3	1	1								
	Charlie's Crack	4	1	2	0.5	0.00072	1	3										
	Gardner's Gut	6	22	3	0.800	0.01693	2	2	2									
	Urenui	6	22	4	0.800	0.01876	1	1	3									1
Aria	Ecch	6	0	1	0	0					6							
Puketiti	All	32	27	5	0.702	0.00795												
	Kuratahi	6	0	1	0	0							6					
	Thunderfall	6	1	2	0.333	0.00048										5	1	
	Grand Canyon	6	0	1	0	0						6						
	Thunderer	2	0	1	0	0						2						
	Greendoor	6	23	2	0.333	0.01106	1					5						
	Unnamed	6	0	1	0	0										6		
	Rimu-Togyp	6	5	3	0.8	0.00385			2					2	2			
All		66	27	12	0.886	0.01501	6	9	8	1	6	13	6	2	2	11	1	1

(c) Molecular variation among mtDNA COI (693bp) of 26 *Neotachycines obliquofasciatus* in six Okinawa, Japan cave population samples.

Area	Cave	n	S	h	Hd	n	Haplotypes (no1-no18)																	
							1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Onna Village	Kabuyagama	5	7	4	0.900	0.00433	1	1	1	2														
Nakijin Village	Asmikaa	2	1	0	0	0												2						
Kunigami Village	Iji Copper mine site	4	11	4	1	0.00818														1	1	1	1	
Ginoza Village	Matsuda-shonyudo	6	3	4	0.800	0.00183									3	1	1							1
Tamagusuku City	Taagaa	4	10	4	1	0.00746					1	1	1	1										
Iheya Village	Kumayaagama	5	0	1	0	0												5						
All		26	64	18	0.954	0.02716	1	1	1	2	1	1	1	1	3	1	1	5	2	1	1	1	1	1

Genetic Diversity and Haplotype Distribution

Although all DNA sequences are for COI from Rhaphidophoridae species the overall final alignment lengths differ, with the shortest in *P. cercalis* (445 bp) and the longest in *N. obliquofasciatus* (1333 bp). For all nine taxa analysed here, DNA sequence alignments within each species were trimmed to the same length. Comprehensive genetic statistics reports for all taxa with available genetic data were generated (Table S2). *Talitropsis sedilloti* exhibited 54 variable sites, resulting in the largest count of haplotypes (30). Conversely, *Pristoceuthophilus* species exhibited the lowest number of haplotypes (6), with 5 segregating sites for *P. celatus* and 10 for *P. cercalis*. The highest Jukes Cantor genetic distance recorded was among *N. obliquofasciatus* from Japan and a *Ceuthophilus* (*C.*) species from Texas, (0.0448, ~4.48%) and the lowest among *D. azami* populations in Italy (0.00569, ~0.57%) (Fig. 2).

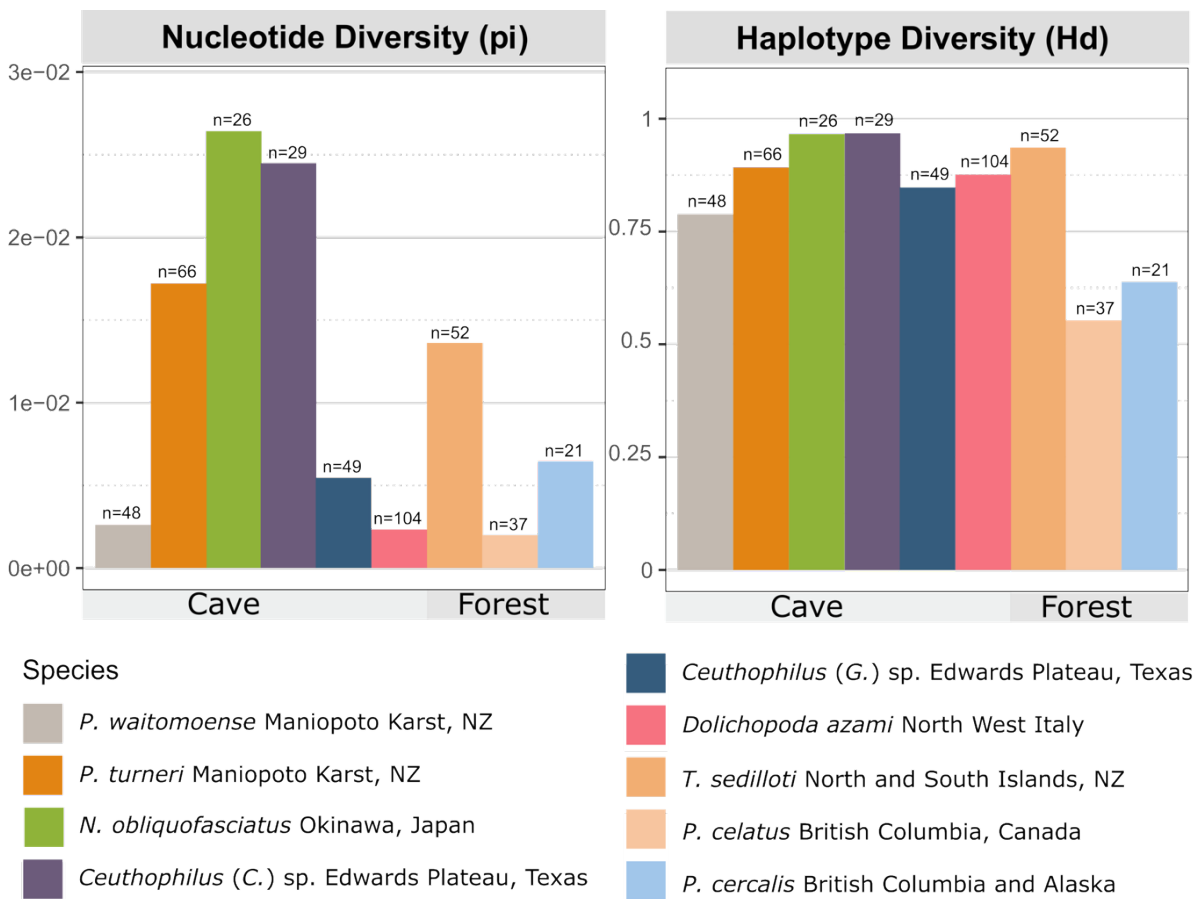


Figure 2. Nucleotide site diversity (π) and haplotype (H_d) diversity among mtDNA COI sequences from populations of nine rhaphidophorid taxa. Genera abbreviations: *Pachyrhamma waitomoense*; *Pallidoplectron turneri*; *Neotachycines obliquofasciatus*; *Talitropsis sedilloti*; *Pristoceuthophilus*

Population Structure of newly sampled Rhaphidophoridae

Within each of the species that coexist in the Maniapoto karst, New Zealand sharing of haplotypes by individuals from different cave systems (area) was uncommon but *pw1* and *pw9* (*P. waitomoense*) were sampled from Puketiti, Piripiri, Waitomo and Mahoenui cave areas, and both *pt1* and *pt3* (*P. turneri*) was found in Waitomo, Puketiti and Mahoenui cave area. Sharing of haplotypes among cave samples within a cave area was observed for five of the eleven *P. waitomoense* haplotypes (*pw1*, *pw4*, *pw7*, *pw8* in Waitomo caves, and *pw9* in Puketiti caves) and for five of the twelve *P. turneri* haplotypes (*pt1*, *pt 2*, *pt3* in Waitomo and *pt16*, *pt10* in Puketiti caves) (Table 2). Thus, diversity within cave samples was low, but variation among cave systems was high (Table 2). Although the level of mtDNA divergence among haplotypes was lower within *P. waitomoense* than within *P. turneri*, both species exhibited geographic structure in the distribution of genetic variation (Fig. 3). On Okinawa, the cave population samples of *N. obliquofasciatus* showed well-structured distinct haplotype groups. While the Kumayaagama cave on Iheya Island and Asmikaa cave had a single distinct haplotype group (*no12* and *no13*), the population samples from caves on the main island comprised three to four distinct haplotypes. Notably, there was no haplotype sharing among individual cave samples of *N. obliquofasciatus* (Table S2; Fig. S2). Analysis of Molecular Variance (AMOVA) among COI sequences of all the sampled taxa indicated significant genetic differentiation both among and within populations with all *p* values < 0.001. *Neotachycines obliquofasciatus* revealed the most genetic variation ($F_{ST} = 0.91$) among the populations when compared to two coexisting species of *P. turneri* ($F_{ST} = 0.62$) and *P. waitomoense* ($F_{ST} = 0.53$). Whereas genetic variation within populations was relatively lower in *N. obliquofasciatus* (9%) than in *P. turneri* (37.57%) and *P. waitomoense* (47.22%). A second AMOVA performed among the cave groups revealed significant genetic variation within groups for both *P. turneri* ($F_{CT} = 0.5614$, *p-value* < 0.001) and *P. waitomoense* ($F_{CT} = 0.4362$, *p-value* < 0.001). These results indicate that genetic differentiation is most pronounced among population samples from different caves of these three species.

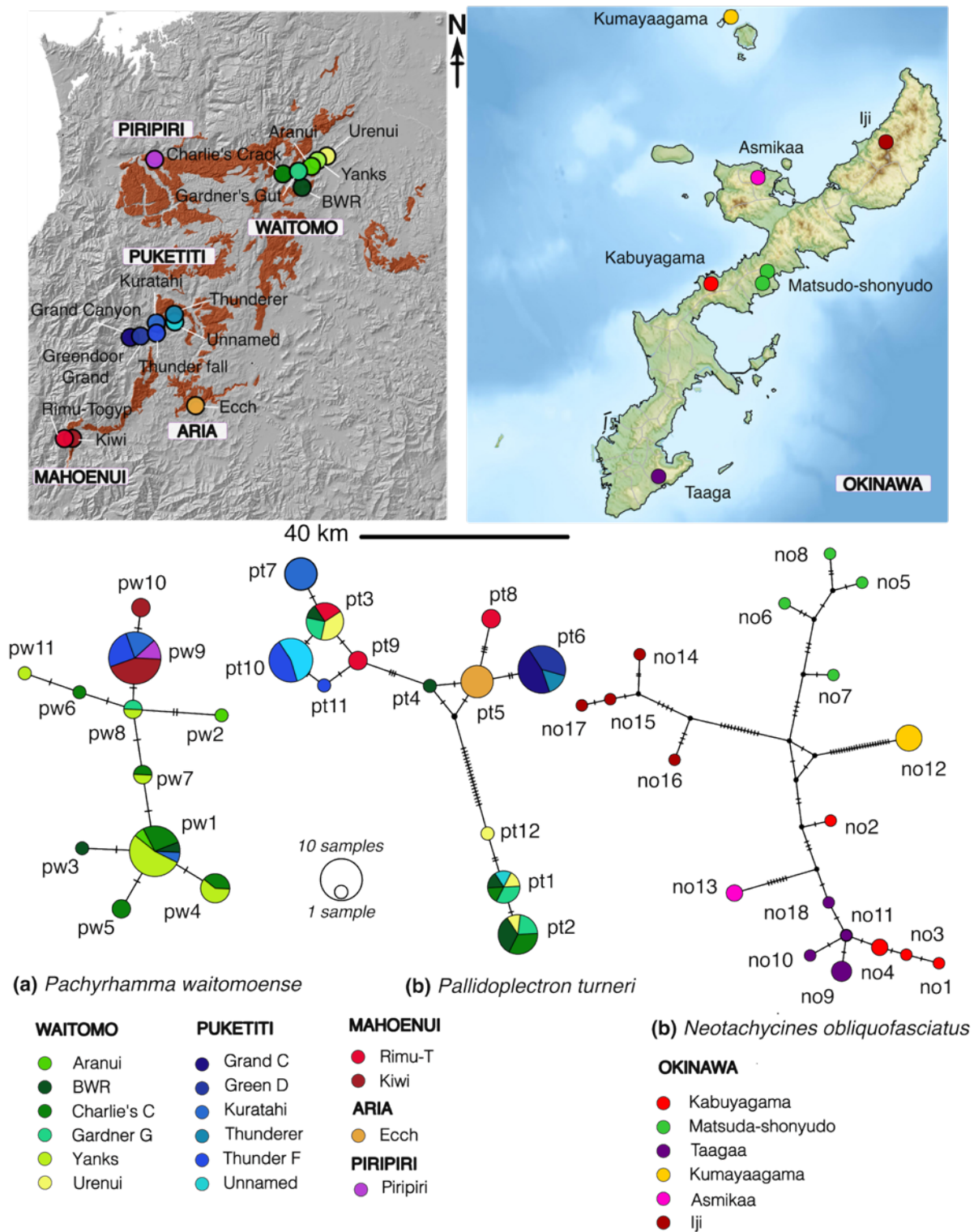


Figure 3. Median-joining networks of mtDNA COI haplotype distribution of three cave dwelling Rhabdophoridae from Maniapoto karst, New Zealand; a) *P. waitomoense* and b) *P. turneri* and Okinawa, Japan; c) *N. obliquofasciatus* based on homologous sequence (693 bp). All the haplotypes are indicated with abbreviations of the taxon and sample locations by different colours. The size of circle is proportional to the number of samples that make up a specific haplotype.

Table 4. Analysis of Molecular Variance (AMOVA) for homologous mtDNA COI (693 pb) haplotypes from *Pachyrhamma waitomoense* (top), *Pallidoplectron turneri* (middle), and *Neotachycines obliquofasciatus* (bottom) as implemented in Arlequin (Excoffier et al., 2005). Statistical significance of variance components in AMOVA was tested with 1023 permutations, and two strategies were tested for *P. waitomoense* and *P. turneri*: (1) one group with all cave population samples, and (2) cave population samples grouped according to geographic area. AMOVA results of taxa with available genetic data are provided in the supplementary table S3.

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-value
1	Among caves	8	28.163	0.60742 Va	52.78	<0.001
	Within caves	39	21.191	0.54337 Vb	47.22	
	Total	47	49.354	1.15079		
	Fixation Index		phiST	0.52783		
2	Among areas	3	24.001	0.77794 Va	56.15	0.0097
	Among caves within areas	5	4.162	0.06427 Vb	4.64	0.00391
	Within areas	39	21.191	0.54337 Vc	39.22	<0.001
	Total	47	49.354	1.38558		
	FSC	0.10576	phiST	0.60784	FCT	0.56146

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-value
1	Among caves	11	220.083	3.25401 Va	59.81	<0.001
	Within caves	54	118.083	2.18673 Vb	40	
	Total	65	338.167	5.44974		
	Fixation Index		phiST	0.59808		
2	Among areas	3	196.085	3.63765 Va	43.62	<0.001
	Among caves within areas	8	106.18	1.98093 Vb	23.75	0.0009
	Within areas	54	146.917	2.72068 Vc	32.62	0.004
	Total	65	449.182	8.33926		
	FSC	0.42133	phiST	0.67375	FCT	0.43621

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-value
1	Among caves	5	209.814	9.54866 Va	88.25	<0.001
	Within caves	20	25.417	1.27083 Vb	11.75	
	Total	25	235.231	10.81949		
	Fixation Index		phiST	0.88254		

Population Structure of previously reported taxa

In the global sampling of Rhaphidophoridae for which genetic data were available, there was some haplotype sharing among samples within taxa, though it was less noticeable in *Ceuthophilus* (*C.*) sp. in North America. Five of the thirty haplotypes in the New Zealand forest species *T. sedilloti* were sampled at more than one location, but none were shared between samples from North and South Islands. Despite small sample size for each population, five of the sixteen haplotypes for *D. azami* were shared. In the forest *Pristoceuthophilus* species, even with few haplotypes identified, haplotype sharing among the individuals of neighbouring samples is evident. In *P. celatus*, haplotype *pc2* is present in all five caves systems (except Pacific Rim NPR), while haplotype *pcr1* in the case of *P. cercalis* is present in individuals of four out of six caves systems. Analysis of Molecular Variance (AMOVA) analysis of all taxa

with available genetic data showed significant genetic variation within populations, with the highest in *D. azami* (97.24%) and the least in *Ceuthophilus* (C.) (49.4%).

Population History

Our mismatch distribution analysis of mitochondrial COI sequences for taxa from Maniapoto karst and Okinawa Island, revealed multimodal distributions for *P. turneri* and *N. obliquofasciatus* (Fig. 4), suggesting these populations are at demographic equilibrium or have experienced long-term stability rather than recent expansion. The observed distributions did not fit the unimodal curve predicted by a sudden expansion model. However, *P. waitomoense* displayed a unimodal distribution curve consistent with a sudden demographic expansion model (Fig. 4). This inference is further supported by the non-significant results for the sum-of-squared deviations (SSD 0.0253, p -value = 0.1960) and Harpending's Raggedness index (rgg = 0.0706, p -value = 0.2960), indicating strong evidence of recent demographic expansion event in *P. waitomoense*.

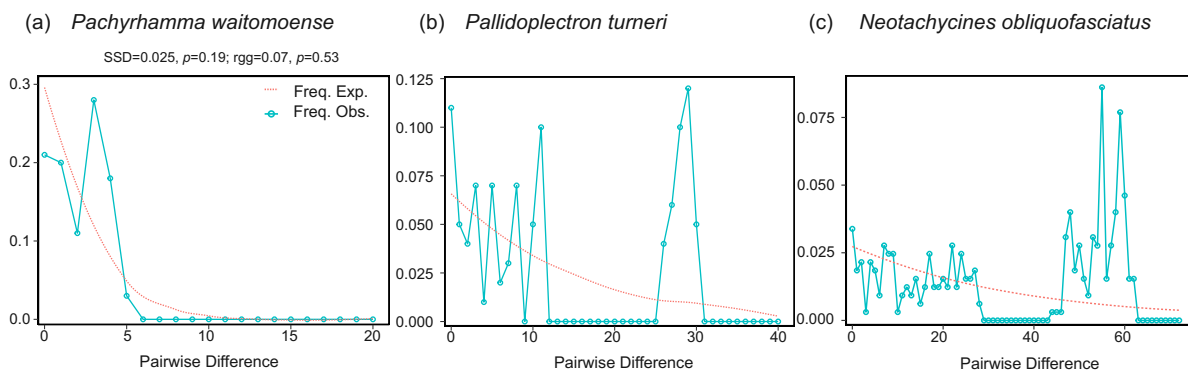


Figure 4. Mismatch distribution of troglophilic camel crickets of Maniapoto karst, New Zealand (a) *Pachyrhamma waitomoense* and (b) (66 individuals) *Pallidoplectron turneri* based on 48 and 66 COI sequences of 806 bp respectively, and (c) *Neotachycines obliquofasciatus* based on 26 COI sequences of 1339 bp. Values of sum-of-square deviation and Harpending's Raggedness index for the unimodal distribution graph of *P. waitomoense* is provided in the top corner. The dashed orange line represents the expected frequency of pairwise nucleotide differences, while the dashed blue line indicates the observed distribution under a model of sudden demographic expansion.

Isolation By Distance

The strong correlation between genetic (Φ_{ST}) and geographic distances in both the Maniapoto species *P. waitomoense* and *P. turneri*, (R^2 values of 0.31; p -value = 0.0125 and 0.59 p -value < 0.001, respectively) indicates a pattern of isolation by distance (Fig. 5). A highly significant association was also observed for *N. obliquofasciatus* from Okinawa Island (R^2 = 0.73, p value

= 0.0083). Significant correlations between genetic and geographic pairwise distances were also detected in British Columbia and Alaska and Central Edwards Plateau Texas taxa *P. cercalis* and *Ceuthophilus* (*G.*), but no evidence of isolation by distance was found for the forest species *P. celatus* and the cave inhabiting *Ceuthophilus* (*C.*) (Fig. 5). Due to small populations samples for *T. sedilloti* and *D. azami* average haplotype genetic distances were calculated instead of Φ_{ST} and analysed their correlation with geographic distance, finding positive associations in both species (Fig. 5).

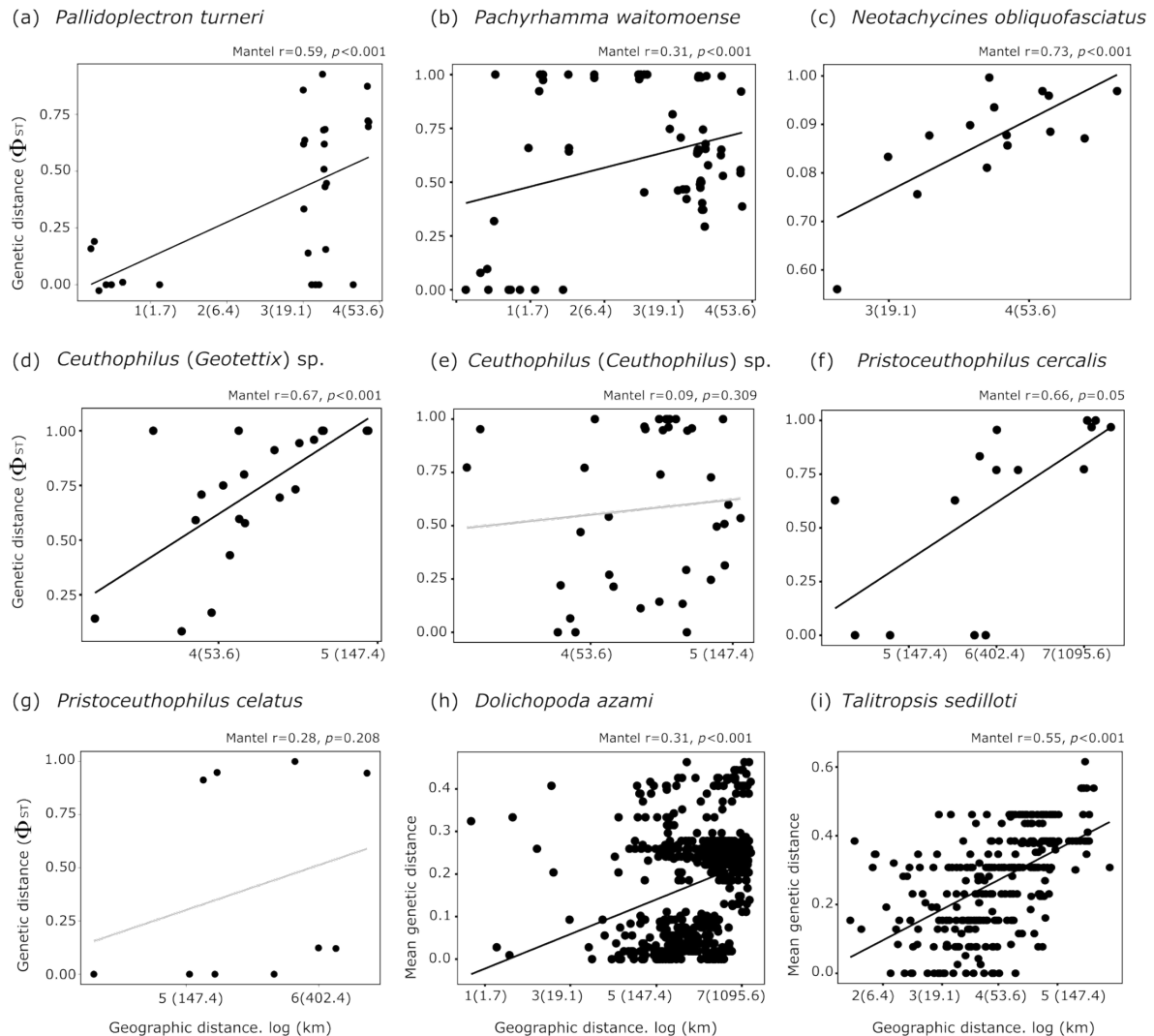


Figure 5. Spatial distribution of genetic diversity in nine species of Raphidophoridae. The trend and results of Mantel tests of the relationship between genetic and geographic distances are shown. Analysis of (a) *P. turneri* and (b) *P. waitomoense* from Waitomo Karst, NZ, (b) *N. obliquofasciatus* from Okinawa Island of Japan, (d) *Ceuthophilus* (*Geotettix*) sp. and (e) *Ceuthophilus* (*Ceuthophilus*) sp. from Texas, USA, (f) *P. cercalis* and (g) *P. celatus* British Columbia and Alaska, used Φ_{ST} , whereas (h) *D. azami* from caves of the Northern Alps of Italy and widely distributed forest species (i) *T. sedilloti* from New Zealand used mean genetic distance (haplotype).

DISCUSSION

The rate of gene flow among cavernicolous populations can be predicted from cave geology, its surface vegetation profile, and the geographic distance separating populations (Caccone, 1985). Populations isolated to individual caves or subterranean networks, are expected to show deeper genetic divergence (Sbordoni et al., 2000).

Maniapoto

The Maniapoto karst area in New Zealand spans 800 km² and is riddled with cave systems (Reid et al., 2012), which prior to the advent of European agriculture (200 years ago) opened into temperate rain forest that would have maintained high humidity and provided ample cool dark habitats (Cumberland, 1941). It is therefore expected that the cave dwelling species *Pachyrhamma waitomoense* and *Pallidoplectron turneri* had connected populations and so show little genetic differentiation among the sampled cave entrance populations. Genetic structure concordant with differentiation in isolation was observed but a signature of isolation by distance in both the species is revealing. Gene flow is not entirely absent, with rare sharing of haplotypes among cave systems detected supporting the IBD inference. Most of the genetic diversity was partitioned among sample sites as might be expected in cave systems or forest habitat. Richards (1962) considered *P. waitomoense* to be primarily cave-dwelling but observed seasonal changes in age structure and feeding habits suggesting that these populations are not highly adapted to cave life (Cesaroni et al., 1997). In addition, *P. waitomoense* individuals were occasionally observed coming out of caves at night to forage in the surrounding forest, despite ample food supply inside the caves (Richards, 1962).

If the sympatric *P. waitomoense* and *P. turneri* have similar population sizes and have responded in parallel to spatial and environmental features of the landscape, comparable levels and patterns of genetic variation are expected. However, different levels of genetic variation were found in these two species. *P. waitomoense* had a lower level of genetic divergence and unimodal mismatch distribution suggesting recent population growth. In contrast, *P. turneri* from same Waikato caves and *N. obliquofasciatus* had higher levels of mtDNA diversity, greater differentiation among cave systems, and a multimodal mismatch distribution suggesting large stable population size with little gene flow among populations. This finding in part, aligned with previous observation of large populations of *P. turneri* in cave entrances in Waikato (Richards, 1961a, 1962) and abundant individuals of *N. obliquofasciatus* at cave's entrances in Okinawa (Sugimoto & Ichikawa, 2003).

Okinawa

On other hand, the lack of haplotype sharing among the cave population samples of *Neotachycines obliquofasciatus* is unexpected as they are considered as habitual troglophiles. Here I found high genetic differentiation among the cave populations although *Neotachycines obliquofasciatus* is not completely restricted to caves. Its biology is not yet studied but life cycle and foraging behaviours at present appeared more seasonal depending on food availability in caves, somewhat similar to some of *Dolichopoda* species. Individuals are also found along streams and large population at the cave entrance. These observations are supported by the signature of isolation by distance that can only arise if there is current or past gene flow among populations.

Global Rhaphidophoridae Taxa

The sympatric cave dwelling camel crickets in Texas also revealed contrasting levels of mtDNA variation although sampled from the same caves (Weckstein et al., 2016). From the same caves in the Central Edwards plateau one lineage (single morphological species) had haplotypes that differed by ~1%, and the other morphological species had haplotypes that differed by ~2.5%. The species considered to be obligate cave dweller had less genetic variation and suggest combination of variation in population size, timing of colonisation and ability to connect via underground tunnels might explain their results as signature of isolation by distance observed (Weckstein et al., 2016). *Pristoceuthophilus cercalis* (3.1%) which is also found in caves showed greater genetic difference compared to the more arboreal *Pristoceuthophilus celatus* (2.3%) (Elliott et al., 2017). This is compatible with the idea the how restricted to cave habitat a taxon is will determining how much populations are differentiated from one another within a species.

In some European *Dolichopoda* species from Tuscany, Italy, significant genetic differentiation and low levels of gene flow among populations were found regardless of the connectivity of limestone environment. However, a trend of isolation by distance (IBD) was also reported, suggesting that historical gene flow likely occurred through migration via forest in the recent past (Allegrucci et al., 1997; Cesaroni et al., 1997). In contrast, cave and forest populations of the North American species, *Ceuthophilus gracilipes*, showed very little genetic differentiation among populations (Cockley et al., 1977). Since *C. gracilipes* is thought to be a relatively sedentary species (highly obligate to cave life), large-scale migration between caves was ruled out as a factor for retaining such high genetic similarity among populations. Instead, it was

proposed that forest populations move among cave populations and act as ‘genetic bridges’, maintaining high enough levels of gene flow to prevent population divergence. In our analysis of IBD across sampled taxa revealed a positive association between genetic and geographic distance, with the exceptions of *Ceuthophilus* (*Ceuthophilus*) sp. and *P. celatus*, where no significant correlation was seen. These contrasting results among cave-dwelling *Ceuthophilus* reflect earlier findings that attributed such variation to differences in underground cave connectivity and the timing of cave colonization (Johnson et al., 2007; Weckstein et al., 2016). For *P. celatus*, low genetic differentiation among populations may be attributed from dispersal capabilities within forests and grasslands that connects sampled cave system. It is also often observed that individuals of both *Ceuthophilus* and *Pristoceuthophilus* are known to forage at night near cave entrances, and utilize surface shelters such as rocks and logs, facilitating gene flow and shaping the observed genetic patterns (Johnson et al., 2007).

Time Scale

Studies in other countries have found that the scale of gene flow between populations of Rhaphidophoridae is dependent on time since cave colonisation. The evidence from speleothem Carbon dating (C^{14} method) and paleomagnetic methods suggests that some Waitomo cave systems in the region are at least 700,000 to 1 million years old (Williams, 1992). Although it may be difficult to estimate the time a cave began to form, the age of terrestrial fossils found in caves (such as moa bones) can potentially give an indicator of when a cave system was opened. This method is perhaps more relevant for estimating time since colonisation by invertebrates. Two tuatara fossil bones collected from Waitomo caves (Flowstone Hole and Ruakuri Cave) have been radiocarbon dated between 1,020 – 1,370 BP years old (Hay et al., 2008). The unique process of karstification in this area means that limestone connectivity and cave age could vary across the Maniapoto Karst System. Since the Pliocene, the rate of karstic development has been affected to a great extent by sea level changes and climatic variations (Williams, 1992). It was then interrupted 0.75 million years ago by deposition of extensive layers of ignimbrite from local volcanic eruptions and again from the late Pleistocene to Holocene by layers of volcanic ash (Williams, 1992). Therefore, the degree of connectivity or isolation of limestone patches probably changed over time. All these processes and events would have affected parts of the karst system differently and, as a result, caves may have been colonised or recolonised by cave wētā at different times. During cold phases of the Quaternary, annual average temperatures were high enough that many plants could still survive in the area (Hendy & Wilson, 1968). This presence of some vegetation in the Waikato region during Pleistocene

means that a constant food source and potential non-cave habitat would have been present outside many caves. This type of environment would have undoubtedly had an affect on the extent to which certain populations were able to adapt to cave life, and also on the amount of suitable environment that was available for migration between caves.

In Okinawa, natural limestone caves are common, and have a record of being colonised by mammals, early humans, molluscs and crustaceans for over 30,000 years (Kawamura et al., 2016). Much younger artificial caves including mine tunnel are also abundant, including many collapse dolines as large as 300m and 10m depth (Gillieson, 2003). Palynology indicates continuous change in subtropical forest from late Pliocene to early Pleistocene, and present day forest started to emerge only 0.8 millions years ago (Fujiki & Ozawa, 2008). This change in vegetation is mostly attributed by climatic change from cooler to warmer temperature due to lower sea level (high salinity) during last glacial periods and rapid tectonic activity in the regions (Fujiki & Ozawa, 2008; Shieh et al., 1997). Dispersal opportunities for cave dwellers may have been influenced by this change in vegetation and climatic fluctuations during Pleistocene. The present patterns of genetic differentiation reflects the historical gene flow that occurred between cave and epigeal populations living in a continuum of wet or mesophilous woody environments (Sbordoni et al., 2000). Highly structured genetic divergence among *N. obliquofasciatus* cave populations suggests population fragmentation due to limited epigeal dispersal that forced the ancestral lineage to be more dependent to cave lifestyle caused by climatic changes that occurred in the Pleistocene.

Conclusion

Three factors might shape population structure of camel crickets: (1) connectivity for the karst landscape, (2) the presence of nearby forest-dwelling populations and (3) adaptation of the population to cave life. For example, the structure and connectivity of limestone karst systems where populations occur was found to be one major determining factor in the level of gene flow occurring among cave-dwelling populations in some North American Raphidophorid species (*Hadenoeus* spp. and *Euhadenoeus* spp.– (Caccone, 1985; Caccone & Sbordoni, 1987). In regions with continuous or interconnected limestone caves, populations are less genetically differentiated because geneflow can potentially occur both above (epigeal) and below ground (hypogean). Whereas in areas of fragmented limestone regions, cave populations constitute genetic isolates, with high structuring even between caves separated by only a few kilometres, suggesting low rates of epigeal dispersal (e.g. *Euhadenoeus insolitus*). Potentially, even in the same cave system, connectedness among populations may reflect different attributes of

individual species (Sbordoni et al., 2000). For instance, some species in subgenus *Ceuthophilus* are frequently found foraging outside caves at night, whereas species in subgenus *Geotettix* are not known to leave their caves. Nevertheless genetic analyses reveal that *Ceuthophilus* exhibits deeper genetic structure than *Geotettix* and no signature of IBD (Johnson et al., 2007; Weckstein et al., 2016). Populations highly adapted to life in caves are characterised by a lack of seasonality and diverse age structure, and specialised feeding habits (Di Russo et al., 1994). In addition, migratory movement out of the cave is infrequent. Populations exhibiting these features were found in natural caves where colonization is thought to be old and where climate and food sources are stable. Caccone, (1985) demonstrated that among cave-dwelling species of crickets inhabiting the same cave systems or the same type of cave system, some exhibit higher levels of gene flow than others. It was thought that of these (*Hadenoeus subterraneus*, *Hadenoeus cumberlandicus*, and *Euhadenoeus puteanus*), the species with low gene flow were more highly adapted (specialised) to cave life than others, causing them to move around between caves less frequently. This pattern of gene flow is further influenced by environmental conditions surrounding caves. In the Mediterranean landscape, *Dolichopoda* population dwelling in caves surrounded by xeric vegetations in coastal habitats are genetically more structured than groups of population living in moist montane forest with higher levels of gene flow (high N_m values) (Allegrucci et al., 1997; Cesaroni et al., 1997).

Several factors may influence the levels of genetic diversity and population structure in different species. These include the ecological requirements or dispersal ability of the species, habitat type and stability, population density or time since colonization of an area. As a result of gene flow and genetic diversity studies, hypotheses have been formed on the nature of speciation across various cave-dwelling Rhabdophorids (Barr & Holsinger, 1985; Cesaroni et al., 1997; Cockley et al., 1977; Howarth, 1993; Sbordoni et al., 1981). The most popular of these, the refugium hypothesis, has been used to explain patterns of little or no gene flow and consequently high genetic divergence within and between some species of cave crickets. It has been suggested that climate change may have driven rhabdophorid speciation if widespread forest-dwelling species are restricted to caves when surface habitat becomes inhospitable and resulting isolation leads to diverge through genetic drift (allopatric speciation) (Allegrucci et al., 2005, 2009; Cesaroni et al., 1997 e.g., Mediterranean). In New Zealand, studies on other arthropods (e.g., grasshoppers) shows a significant phylogeographic structure that predates the Pleistocene, indicating their survival across all or part of the glacial period in multiple distinct refugia (Trewick et al., 2011; Wallis & Trewick, 2009). Alternatively, as proposed for other cave

arthropods (Barr & Holsinger, 1985; Howarth, 1993; Schilthuizen et al., 2005), cave-dwelling populations might diverge from conspecific bush-dwelling populations even with gene flow occurring between the two populations, in response to contrasting environmental conditions (Schilthuizen et al., 2005).

Determining which factors or processes may have produced the patterns of genetic diversity was not the intention of this work, but hypotheses for further investigation can be formed based on information in the literature. My single locus and population sampling provide overall insights into genetic structures and isolation by genetic distances among cave associated camel crickets' populations. However, extensive future sampling across multiple loci and populations has the potential to increase our understanding of the evolutionary role of ecological, environmental and geographical factors in determining continuous or disjunct distributional patterns. Based on my genetic evidence of infrequent epigean and hypogean dispersal, differences in habitat adaptation appear to be more important in shaping population structure in these cave dwellers than the availability of dispersal routes. The absence and/or rare sharing of gene material among cave samples within these small geographic scales, combined with increasing habitat fragmentation and rapid climate change driven by anthropogenic activities, will lead to small and fragmented relict populations with limited capability to adapt to selective forces emerging from environmental pressure.

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SUPPLEMENTARY INFORMATION

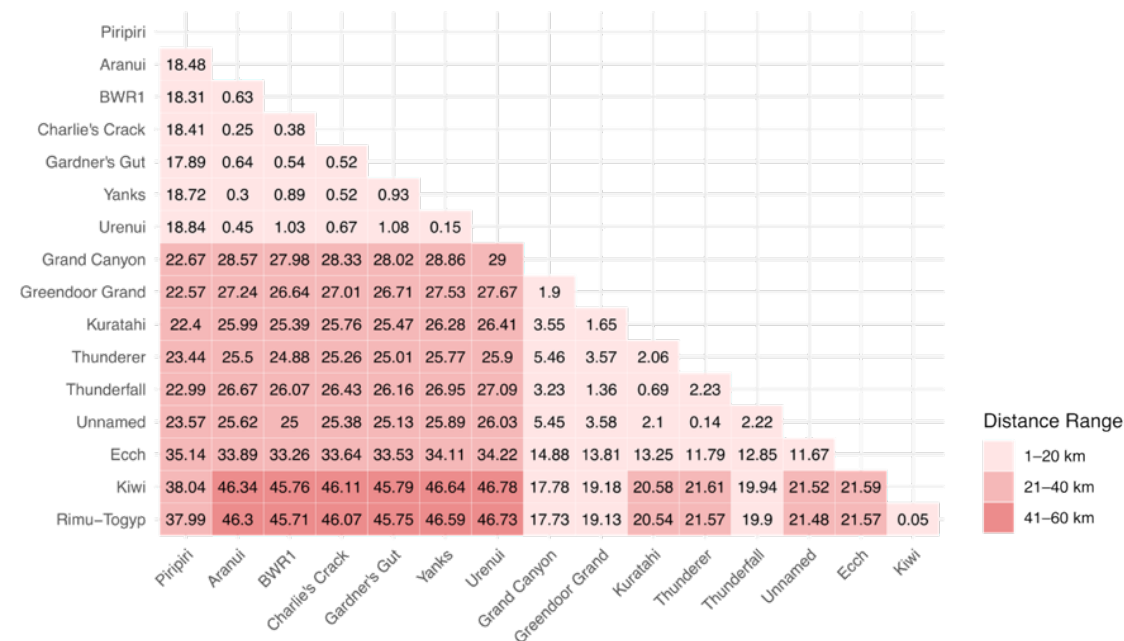


Figure S1. Pairwise geographic distance among 16 caves within five cave areas estimated based on the shortest distance between two points on an ellipsoid.

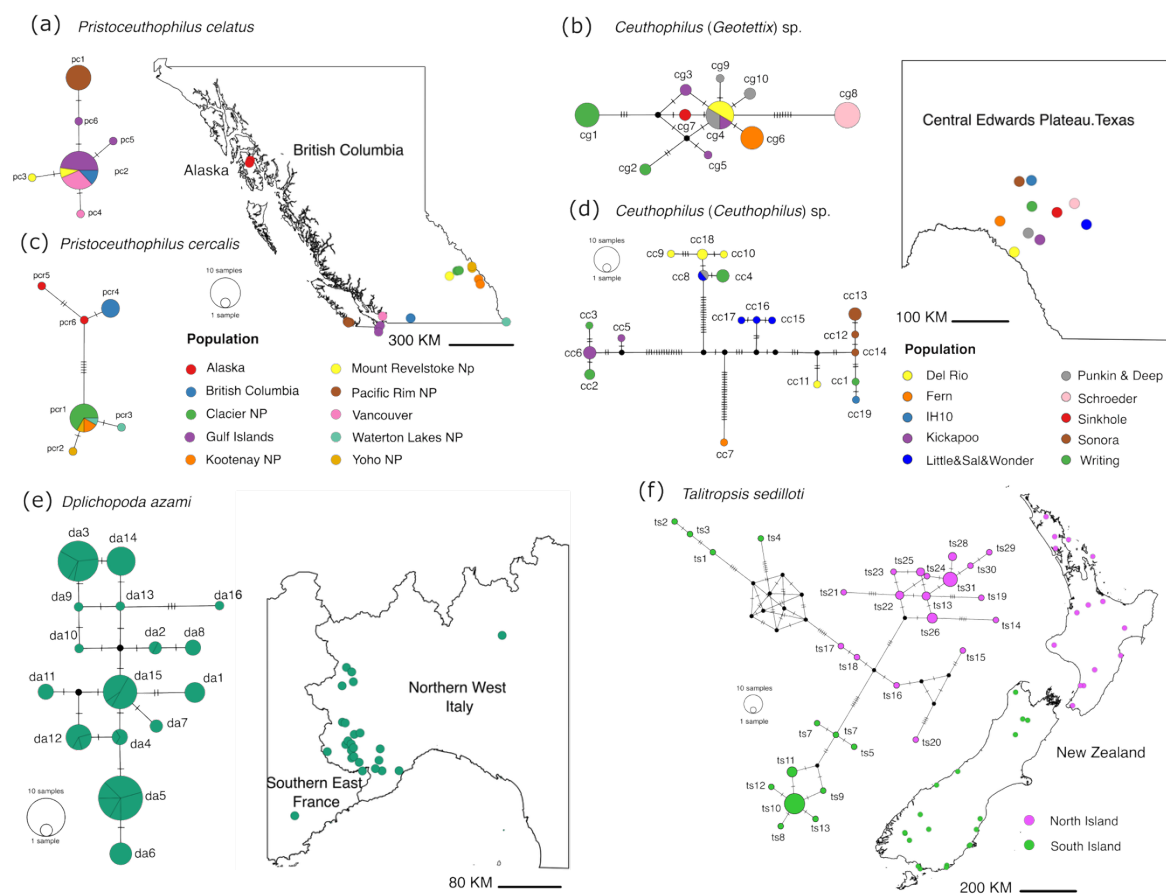


Figure S2. Median-joining networks showing the arrangement of the COI haplotype distribution of the sampled taxa (based on available genetic data) with sampled location maps to the right of haplotype networks. All the haplotypes are indicated with abbreviations of the taxon and sample locations by different colours (except for *D. azami* and *T. sedilloti*). The size of circle is proportional to the number of samples that make up a specific haplotype. The small grey circles represent lost or unsampled haplotypes. The sequence alignment length is based on the information provided in table 1.

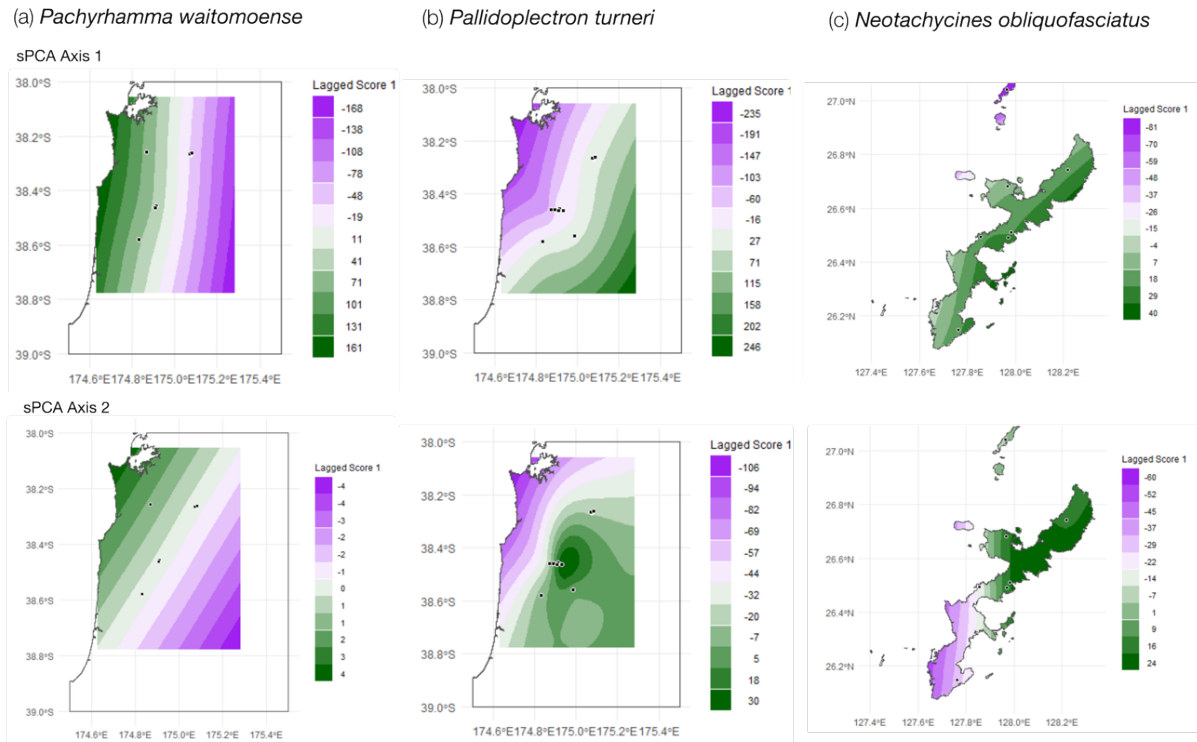


Figure S3. Interpolation of first and second axes of spatial PCA on mtDNA haplotypes. (a) Interpolation of first axis of PCA of pronotum shape. The finding of this analysis was not included due to small sample size (no-spatial genetic variation or very subtle spatial variation was only found). (Brief method used) The spatial relationships among sampling sites were modelled using a Gabriel graph connection network based on pairwise geographic distances. Spatial principal component analysis (sPCA) was performed using the *ade4* R package (Jombart, 2008) to quantify and visualise global patterns of spatial genetic structure (haplotype), emphasising the first two positive principal axis values. Spatial patterns in sPCA scores were further visualised and interpreted through interpolation, generating continuous genetic surfaces across the sampled landscape to evaluate spatial gradients and potential barriers to gene flow.

Table S1. Pairwise genetic distance (phiST) among sampled caves for all newly sequenced taxa computed using Arlequin with 1000 number of permutations.*(a) Pachyrhamma waitomoense*

		Piripiri	BWR	Charlie's Crack	Gardner's Gut	Aranui	Yanks	Kuratahi	Thunder fall	Kiwi
Piripiri	Piripiri	0.000								
Waitomo	BWR	0.857	0.000							
	Charlie's Crack	0.619	-0.026	0.000						
	Gardner's Gut	1.000	0.600	0.333	0.000					
	Aranui	0.333	0.000	0.159	0.000	0.000				
	Yanks	0.636	0.011	-0.053	0.363	0.190	0.000			
Puketiti	Kuratahi	-0.263	0.508	0.431	-0.200	0.155	0.447	0.000	Puketiti	
	Thunder fall	0.000	0.927	0.681	1.000	0.619	0.684	0.000	0.000	
Mahoenui	Kiwi	-0.194	0.874	0.722	0.682	0.696	0.717	0.139	-0.006	0.000

(b) Pallidoplectron turneri

		BWR!	Charlie's Crack	Gardner's Gut	Urenui	Ecch	Kuratahi	Thunder fall	Grand Canyon	Thunderer	Green door	Unnamed	Rimu-Togyp
Waitomo	BWR!	0.000											
	Charlie's Crack	0.079	0.000										
	Gardner's Gut	-0.183	0.093	0.000									
	Urenui	-0.090	0.320	-0.119	0.000								
Aria	Ecch	0.602	0.991	0.617	0.480	0.000	Aria						
Puketiti	Kuratahi	0.636	0.992	0.617	0.435	1.000	0.000					Puketiti	
	Thunder fall	0.623	0.982	0.605	0.420	0.971	0.923	0.000					
	Grand Canyon	0.651	0.992	0.662	0.552	1.000	1.000	0.979	0.000				
	Thunderer	0.469	0.983	0.484	0.336	1.000	1.000	0.965	0.000	0.000			
	Greendoor	0.361	0.741	0.383	0.262	0.258	0.635	0.613	0.000	-0.304	0.000		
	Unnamed	0.636	0.992	0.617	0.435	1.000	1.000	0.000	1.000	1.000	0.635	0.000	
Mahoenui	Rimu-Togyp	0.521	0.912	0.509	0.299	0.667	0.500	0.449	0.778	0.651	0.400	0.500	0.000

(c) *Neotachycines obliquofasciatus*

	Kabuyagama	Taagaa	Matsuda_shonyudo	Kumayaagama_5	Asmikaa	Iji
Kabuyagama	0.000					
Taagaa	0.298	0.000				
Matsuda_shonyudo	0.723	0.814	0.000			
Kumayaagama	0.951	0.978	0.918	0.000		
Asmikaa	0.764	0.891	0.785	1.000	0.000	
Iji	0.829	0.882	0.752	0.926	0.848	0.000

Table S2. Sampling statistics and genetic characteristics of haplotype diversity among population sampled of taxa with available genetic data. Abbreviations used in the table are as following: Number of individuals, number of Segregating sites, number of haplotypes, Haplotype diversity, nucleotide diversity π (Nei, 1987). Statistics were calculated using DnaSP (Librado & Rozas, 2009).

(a) *Ceuthophilus (Ceuthophilus) sp.*

	Locality	Latitude	Longitude	Cave/Forest/Mine site	(bp)	n	S	h	Hd	Pi
1	Edwards	30.15446296	-100.576279	Writing on the Rocks Cave	850	7	45	4	0.81	0.02662
2	Kinney	29.61759674	-100.427828	Kickapoo Cavern	850	4	3	2	0.5	0.00176
3	Val Verde	29.91749745	-101.171764	Fern Cave	850	1				
4	Val Verde	29.41862703	-100.905587	cave near Del Rio	850	5	30	4	0.9	0.01412
5	Sutton	30.5551726	-100.811996	Caverns of Sonora	850	5	2	3	0.7	0.00118
6	Kerr	30.20060426	-99.7824457	Schroeder Bat Cave	850	1	0	1		
7	Real	29.71958526	-99.674181	Little Dry Frio Cave	850	4				
8	Real	29.7189126	-99.6626279	All the Wonders and Joys Cave	850					
9	Bandera	29.86219384	-99.5598073	near Haby Salamander Cave	850		2	2	1	0.00235
10	Edwards	29.72494036	-100.644614	Punkin Cave	850	1				
11	Sutton	30.57191435	-100.585043	IH 10 Cave	850	1				
	All					29	26	19	0.968	0.02447

(b) *Ceuthophilus (Geotettix) sp.*

	Locality	Latitude	Longitude	Cave/Forest/Mine site	(bp)	n	S	h	Hd	Pi
2	Edwards	30.15446296	-100.576279	Writing on the Rocks Cave	850	11	6	2	0.327	0.00231
3	Kinney	29.61759674	-100.427828	Kickapoo Cavern	850	5	3	3	0.8	0.00165
4	Val Verde	29.91749745	-101.171764	Fern Cave	850	8	0	1	0	0
5	Val Verde	29.41862703	-100.905587	cave near Del Rio	850	6	0	1	0	0
7	Edwards	30.05740768	-100.109008	Devil_s Sinkhole	850	2	0	1	0	0
8	Kerr	30.20060426	-99.7824457	Schroeder Bat Cave	850	10	0	1	0	0
13	Edwards	29.72494036	-100.644614	Punkin Cave	850	3	2	3	1	0.00157
16	Edwards	29.72240318	-100.64112	Deep Cave	850	4	1	2	0.5	0.00059
	All						18	10	0.848	0.00545

(c) *Dolichopoda azami*

	Locality/Cave	Latitude	Longitude	(bp)	n	S	h	Hd	Pi
1	Taragnina Cave	44.12119444	8.172058333	1409	5	0	1	0	0
2	Balmarossa Cave	44.42694444	7.292280556	1409	1				
3	Bandito Cave	44.26835278	7.390294444	1409	1				
4	Bornadel Servais Cave	45.32434167	7.304233333	1409	1				
5	Besta Cave	44.11845556	7.568	1409	2	0	1	0	0
6	Bucodelle Locuste Cave.	44.544225	7.433641667	1409	2	0	1	0	0

Table S2 (c) *Dolichopoda azami* (cont.).

	Locality/Cave	Latitude	Longitude	(bp)	n	S	h	Hd	Pi
7	Bucodel Maestro Cave	44.68645833	7.2375	1409	6	0	1	0	0
8	Bossea Cave,	44.242075	7.840855556	1409	2	0	1	0	0
9	Bucodel Partigiano Cave	44.468525	7.330411111	1409	7	0	1	0	0
10	Mine close to Chauves-souris Cave	43.59617778	6.451947222	1409	3	0	1	0	0
11	Chiabrano Cave.	44.67707222	7.283702778	1409	6	0	1	0	0
12	Villar Focchiardo Cave,	45.10969722	7.232908333	1409	8	0	1	0	0
13	Farout Cave	44.43256667	7.382572222	1409	2	1	2	1	0.00071
14	Orso Cave	44.11971944	7.891166667	1409	3	0	1	0	0
15	Partigiani Cave	44.560175	7.529733333	1409	8	0	1	0	0
16	Viozene	44.14496389	7.781313889	1409	1				
17	Gaiola Cave.	44.34291389	6.988113889	1409	3	0	1	0	0
18	Borna Maggiore del Pugnetto Cave	45.27162778	7.391166667	1409	11	1	2	0.182	0.00013
19	Â Rittana Cave	44.35768889	7.385291667	1409	3	1	2	0.667	0.00047
20	Bandit o hypogeum	44.28535278	7.426730556	1409	7	0	1	0	0
21	Vernante hypogeum	44.21837778	7.513894444	1409	6	0	1	0	0
22	Vallone Sabeneastern hypogeum	44.38764444	7.421141667	1409	4	0	1	0	0
23	Dronera Cave	44.375475	7.860647222	1409	6	2	2	0.333	0.00047
24	Valdella Torre Mine	45.1572	7.418125	1409	5	0	1	0	0
25	Lacca Selva Cave	45.697625	9.872913889	1409	1				
					104	13	16	0.876	0.00232

(d) *Pristoceuthophilus celatus*

	Locality	Latitude	Longitude	Cave/Forest/Mine site	(bp)	n	S	h	Hd	Pi
1	British Columbia, Canada	48.673	-123.429	Gulf Islands National Park Reserve	513	4	0	1	0	0
2	British Columbia, Canada	48.794	-123.31	Gulf Islands National Park Reserve	513	8	3	3	0.464	0.00148
3	British Columbia, Canada	48.5197	-123.43	Victoria	513	1				
4	British Columbia, Canada	51.022	-118.207	Mount Revelstoke National Park	513	3	1	2	0.667	0.0013
5	British Columbia, Canada	49.301	-123.14	Vancouver	513	8	1	2	0.25	0.00049
6	British Columbia, Canada	49.048	-125.704	Pacific Rim National Park Reserve	513	2	1	2	1	0.00195
7	British Columbia, Canada	49.027	-125.647	Pacific Rim National Park Reserve	513	2	0	1	0	0
8	British Columbia, Canada	49.034	-125.554	Pacific Rim National Park Reserve	513	1				
9	British Columbia, Canada	49.044	-125.684	Pacific Rim National Park Reserve	513	2	0	1	0	0
10	British Columbia, Canada	49.071	-125.753	Pacific Rim National Park Reserve	513	1				
1	British Columbia, Canada	49.013	-125.656	Pacific Rim National Park Reserve	513	2	0	1	0	0
1	British Columbia, Canada	49.216	-121.068	British Columbia	513	3	0	1	0	0
2	Canada									
	All					37	5	6	0.553	0.00199

(e) *Pristoceuthophilus cercalis*

	Locality	Latitude	Longitude	Cave/Forest/Mine site	(bp)	n	S	h	Hd	Pi
1	British Columbia	49.216	-121.068	British Columbia	493	5	0	1	0	0
2	Alaska	55.981	-132.89	Alaska	493	1				
3	Alaska	55.894	-132.944	Alaska	493	1				
4	British Columbia	50.885	-116.052	Kootenay National Park	493	1				
5	British Columbia	50.675	-115.931	Kootenay NP	493	1				
6	British Columbia	51.365	-116.528	Yoho National Park	493	1				
7	British Columbia	51.443	-116.542	Yoho NP	493	1				
8	British Columbia	51.243	-117.649	Glacier National Park	493	7	0	1	0	0
9	British Columbia	51.26	-117.49	Glacier National Park	493	1				
10	Alberta	49.059	-114.013	Waterton Lakes National Park	493	2	1	2	1	0.00203
	All					21	10	6	0.638	0.00645

(f) *Talitropsis sedilloti*

	Locality	Latitude	Longitude	(bp)	n	S	h	Hd	Pi
1	N.I., HawkesBay, Mohi Bush	-39.6646	176.97259	744	2	4	2	1	0.00538
2	N.I., Northland	-35.14469	173.76049	744	2	1	2	1	0.00134
3	N.I., TeUrewera, L. Waikaremoana, Black Beech Track, and Hinerau Walk	-38.76978	177.13759	744	4	8	3	0.833	0.0056
4	N.I., Mt Taranaki, East-Taranaki, Patea Track	-39.32576	174.10749	744	4	3	3	0.833	0.00202
5	N.I., Levin, Tararua Ra., Makahika Lodge	-40.64688	175.3785	744	1				
6	N.I., Manawatu, Levin, Papaitonga Reserve	-40.64336	175.23338	744	1				
7	N.I., Coromandel, Cuvier Island	-36.43908	175.77056	744	3	0	1	0	0
8	N.I., Manawatu, Pohangina Valley, Camp Rangi Woods	-40.12979	175.85097	744	1				
9	N.I., Waikato, Waitomo Caves, Short Bush Walk	-38.26999	175.08799	744	1				
10	N.I., Waikato, Te Awamutu, Maunga tauturi	-38.01911	175.576	744	1				
11	N.I., BOP. Mangatoi, Otanewainuku Forest, Rimu Tr.	-37.90512	176.2048	744	1				
12	N.I., Northland, Hukatere	-36.17831	174.16802	744	1				
13	N.I., Lady Alicels land	-35.89049	174.72952	744	1				
14	N.I., Northland, Whatitiri Scenic Res.	-35.79313	174.13314	744	1				
15	N.I., Wellington, Eastbourne	-41.29714	174.90024	744	1				
16	N.I., Northland	-35.14469	173.76049	744	1				
	All (Just North Island)				26	30	17	0.951	0.00598
17	S.I., Dunedin, Frasers Bush	-45.86176	170.45827	744	1				
18	S.I., Otago, Leith Valley, Dunedin	-45.83532	170.49487	744	2	1	2	1	0.00134
19	S.I., Nelson, Nelson Lakes, Mt. Roberts, Carpark	-41.83425	172.81356	744	1				
20	S.I., Nelson Lakes NP, Lake Rotoroa, loop Track	-41.79421	172.59951	744	1				
21	S.I., Te Anau, Rainbow Reach	-45.45529	167.69441	744	1				
22	S.I., Fiordland NP, Te Anau, Kepler Track	-45.43394	167.68473	744	2	1	2	1	0.00134
23	S.I., Catlins, Matai Falls	-46.49868	169.49212	744	1				

Table S2 (f) *Talitropsis sedilloti* (cont.).

	Locality	Latitude	Longitude	(bp)	n	S	h	Hd	Pi
24	S.I., Otago, Catlins Coast, Papatowai	-46.56906	169.47114	744	1				
25	S.I., Southland, Takitimu Ra., Pinchester Bush	-45.68278	167.88375	744	3	0	1	0	0
26	S.I., Invercargill, Bluff Scenic Res.	-46.61444	168.34502	744	2	1	0	0	0
27	S.I., Invercargill, Otarara Scenic Res.	-46.52892	168.25739	744	1				
28	S.I., Kahurangi NP, Golden Bay, Start of Heaphy Track	-40.86076	172.45167	744	3	6	3	1	0.00538
29	S.I., Lake Wakatipu, TeKere Haka Reserve	-45.32489	168.71396	744	1				
30	S.I., Otago, Queenstown, Kinloch	-44.84283	168.3496	744	1				
31	S.I., Otago, Hampden, Kurinui	-45.33013	170.76286	744	1				
32	S.I., Central Otago, nr. Beaumont	-44.98098	170.9261	744	1				
33	S.I., West Coast, Lake Matheson	-43.43946	169.96273	744	1				
34	S.I., Lewis Pass NP, Lake Daniells Track	-42.3033	172.28494	744	1				
35	S.I., Westland, Haast River	-43.88322	169.04867	744	1				
	All (Just South Island)				26	36	13	0.788	0.00957
	All (Both North and South Island)				52	54	30	0.936	0.01361

Table S3. Analysis of Molecular Variance (AMOVA) for mtDNA COI haplotypes of sampled taxa (taxa with available genetic data) as implemented in Arlequin (Excoffier et al., 2005). Statistical significance of variance components in AMOVA was tested with 1023 permutations.(a) *Ceuthophilus* (*Ceuthophilus*) sp.

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
1	Among populations	8	179.82	5.52620 Va	49.4	<0.001
	Within populations	20	113.214	5.66071 Vb	50.6	
	Total	28	293.034	11.18692		
	Fixation Index	phiST	0.49399			

(b) *Ceuthophilus* (*Geotettix*) sp.

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
1	Among populations	17	282.123	3.34103 Va	94.03	<0.001
	Within populations	73	15.482	0.21208 Vb	5.97	
	Total	90	297.604	3.55311		
	Fixation Index	phiST	0.94031			

(c) *Pristoceuthophilus celatus*

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
1	Among populations	4	14.558	0.51373 Va	82.91	<0.001
	Within populations	32	3.388	0.10587 Vb	17.09	
	Total	36	17.946	0.6196		
	Fixation Index	phiST	0.82913			

(d) *Dolichopoda azami*

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
1	Among populations	24	164.604	1.66639 Va	97.24	<0.001
	Within populations	79	3.742	0.04737 Vb	2.76	
	Total	103	168.346	1.71377		
	Fixation Index	phiST	0.97236			

(e) *Pristoceuthophilus cercalis*

Test	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
1	Among populations	5	28.952	1.76786 Va	92.99	<0.001
	Within populations	15	2.00	0.13333 Vb	7.01	
	Total	20	30.952	0.6196		
	Fixation Index	phiST	0.92987			

CHAPTER FIVE: Thesis Discussion

Evolution and climate change on mountains: Bhutan as an East Himalaya hotspot

Bhutan is a Biodiversity Hotspot

The Himalaya is a major mountain ecosystem that hosts many of the world's key biodiversity hotspots (Myers et al., 2000), but the taxa here are rarely included in ecological and evolutionary studies. Here I begin to address this absence of sampling and lack of study of the insects from this region (Avisé, 2000). The Himalaya spans an area of almost 600,000 km² in which political borders of China, India, Bhutan, Nepal, and Pakistan occur; a reflection of the extreme conditions in these mountains that also define climatic and biogeographic boundaries. The region is distinguished by a distinctive flora and fauna with high levels of species endemism (Myers et al., 2000), and numerous critical ecoregions due to its varied landscapes, vegetation, and climate. Four of the world's 36 recognized biological diversity hotspots are here: the Himalaya, Indo-Burma, the mountains of southwest China and Central Asia (Olson et al., 2001; Olson & Dinerstein, 2002), 60 of the 200 global ecoregions, and 330 important bird and biodiversity Areas (Kotru et al., 2020).

Within this large and complex landscape is the small nation of Bhutan (~38,394 km²) in the heart of southern slope of the Himalaya between the cold arid Tibetan Plateau to the north and the wet tropical lowlands of Bengal (Royal Government of Bhutan, 2022). To the west and east of this continental narrowing are the massive regions of India and Malayan Peninsula respectively. The mountains are mostly steep with deep narrow valleys crisscrossed by fast-flowing streams and major rivers. Due to this geography, extreme elevation variation, and varied ecological conditions, it is home to a substantial amount of biodiversity. Bhutan's position at the nexus (Sino-Japanese) of the Palearctic and Oriental biogeographical regions reflects a complex ecosystem that blends biodiversity of South China and Indo-Burma and so constitutes a critical ecotone region (Holt et al., 2013; Myers et al., 2000).

Elevation Gradient and Species Richness

In biodiversity research proxies are essential tools because species richness cannot be measured directly for all organisms. Ecologists and conservation biologists rely on proxies such as particular plant or insect families to infer broader biodiversity patterns.

(Brunbjerg et al., 2018; Gotelli & Colwell, 2001; Lino et al., 2024; Ritter et al., 2019). The camel crickets of the family Rhaphidophoridae are an example of a taxonomic group that might serve as a suitable proxy for studying species richness in complex mountain ecosystems. Though well represented in the region these insects are understudied (Dorji & Trewick, 2025 Chapter 1). I applied a combination of methods that would be suitable for many biological groups to explore local diversity, biogeographic and phylogenetic context, and population-level processes that underpin diversification and maintenance of biodiversity. Future steps will be modelling of species distributions using climatic and other variables applicable across the landscape (Hof et al., 2024; Sharma et al., 2025). My study on camel crickets of Bhutan revealed family prevalence/occurrence widely across elevation ranging from 150 m to 3,000 m asl, which is partly covered by snow in winter (Dorji & Trewick, 2025 Chapter 1; Dorji et al., 2025b Chapter 2). The impact of elevation gradient on camel cricket species richness and its diversification is unknown and there remains scope for generating new knowledge using research applications from this study following completion of this study (for example Sun et al., 2020).

Bhutan is characterized by a steep elevation gradient, complex topographic features, and diverse land cover (Royal Government of Bhutan, 2022). Examining species richness across elevation gradients provides the opportunity to study the potential drivers of spatial variation in biodiversity. Variables such as temperature, area, and elevation are frequently correlated with estimates of species richness and the changing composition of ecological assemblages (Grytnes, 2003; Lomolino, 2001; Yu et al., 2013). In ecological studies, three different patterns have been documented: monotonic, unimodal, and multimodal (Grytnes & Vetaas, 2002; Kluge et al., 2017; Sun et al., 2020a), of which unimodal patterns are the most prevalent in mountain systems. Nearly half of all elevation gradients of species richness show a mid-elevation peak, despite different organisms having their maximum number of species at different elevations (Graham & Duda, 2011). There are many studies explaining elevation diversity patterns involving different taxa and regions (Kluge et al., 2017; McCain, 2005; Quintero & Jetz, 2018), but clear conclusions about the patterns and mechanisms of invertebrates in mountains remain elusive. Some biologists have suggested that unimodal relationships indicate dynamic non-equilibrium process at play making it important to have a better understanding of both the distribution patterns and the variables that produce and maintain biodiversity in montane ecosystems (Graham & Duda, 2011).

Species Diversity and Endemism

More than half of world's camel crickets' diversity (251 species) is found in Asian tropical and temperate regions within the three lineages Rhaphidophorinae, Aemodogryllinae, and Anoplophilinae (Cigliano et al., 2023). The rapid rate of new species discovery over the past two decades has tripled the known number of species, highlighting Asia as a diversity hotspot for Rhaphidophoridae. However, species representation across this region remains uneven, with China alone accounting for 55% of the total biodiversity and a notable lack of records from the western Himalayas and Indian subcontinent, creating a substantial gap in the biogeographic puzzle of Rhaphidophoridae on the Asian continent. In Bhutan, despite elevation variation, only eleven camel cricket species are known (Dorji et al., 2025b Chapter 2; Ingrisch, 2002; Wurmli, 1973). Six species are from the Northeast Asian subfamily Aemodogryllinae and five from the Southeast Asian subfamily Rhaphidophorinae, an example of biogeographic regions intersecting in Bhutan. This however, constitutes a fraction of the species diversity that is thought to exist in tropical and subtropical forests, as vast areas of Bhutan's territory remain insufficiently studied (Dorji & Trewick, 2025 Chapter 1). Such studies pioneer valuable baseline for future exploration of Rhaphidophoridae with detailed identification keys for all known species from Bhutan based on the species found in the region illustrated. However, our understanding of the true diversity and geographical distributions of camel crickets remain limited, emphasizing need for systematic exploration of caves, cloud forest and alpine mountains within national parks and reserves to add to the existing knowledge in the understanding of broader questions of evolution process of this wingless orthoptera from extreme physiographical processes.

The number of taxa, specifically Rhaphidophorinae (Bian et al., 2017; Bian & Shi, 2016; Dawwrueng et al., 2020) and Aemodogryllinae (Dorji et al., 2025b Chapter 2; Gorochoy & Storozhenko, 2015, 2019; Qin et al., 2018; Wang et al., 2019; Zhu & Shi, 2018) from temperate and tropical of Asia has increased and will undoubtedly increase in near future as many lineages awaits for discoveries. Owing to expanding study of camel crickets in the region, the taxonomic status of morphologically described species has become increasingly complex, even at the generic level of classifications. Further corroborated by the presence of high degree of morphological similarity and cryptic diversity particularly within Aemodogryllinae, making it more challenging to delimitate based solely on morphological characteristics. Use of molecular techniques in such circumstances help resolve systematics ambiguity yet integrative approach combining both morphology and molecular methods remain rare and challenging in the region.

Within this family, numerous molecular based studies attempts have been made in other region to establish an agreement on standard subfamily, and generic groupings (Allegrucci & Sbordoni, 2019; Beasley-Hall et al., 2024; Dowle et al., 2024; Garzia et al., 2025; Hegg et al., 2019, 2022; Kim et al., 2024). No comprehensive phylogenetic consensus based on molecular DNA has been achieved for the Asian lineage. To date, only one study has examined the phylogeny of taxa within the genus *Diestramima*, supporting the monophyly of genus but not the traditional classification system of three subgenera, an inconsistency also reported in this study (Dorji et al., 2025b Chapter 2) including discrepancies within existing genera *Diestrammena* and *Tachycines* (Dorji et al., 2025a Chapter 3). Discrepancies in Asian lineage of camel crickets is inevitable due to heavy reliance on morphologic description in past, an integrative approach integrating both molecular and morphological characteristics and including multiple loci (mitochondrial and nuclear) for molecular analyses would assist reduce taxonomic ambiguity and in narrowing down morphological characters that are phylogenetically informative for taxonomist and useful for identification by stakeholders (field researchers).

Phylogeny and Phylogeography

Phylogenetic analyses provide a framework for understanding how evolutionary history shapes present-day patterns of species diversity (Avise, 2000). By applying these methods to biodiversity hotspots, researchers can reveal both the drivers of speciation, and which lineages contribute high phylogenetic diversity (and conservation value). The reconstruction of phylogeny is an essential first step towards understanding the origin and diversification of organism. In the case of Rhaphidophoridae, accurate inferring of the timing of phylogenetic relationship has always been challenging due to lack of reliable fossils. The present endemic distribution of Rhaphidophoridae is hypothesize as a result of combination of both dispersal and vicariance as an explanation for biogeography patterns (Allegrucci & Sbordoni, 2019). Family phylogenetic study based on secondary calibration of fossil of Ensifera suggest, Northern hemisphere Rhaphidophoridae (Aemodogryllinae, Rhaphidophoridae and Dolichopodainae) divergences concordant to palaeogeological events of Beringia and southern (Macropathinae) indicating dispersal and vicariance events when the region was connected and separated in biogeographic history (Kim et al., 2024). The study suggests early Cretaceous divergence (133 mya) of eastern Laurasia lineage from Macropathinae from northern Asia to Southeast Asia through multiple recolonizations events. The only phylogenetic study of Asian lineage is that of Zhu et al., (2022) who inferred the timing of origin of single genus

Diestramima from China at around ~20 mya. My fossil calibrated molecular clock analysis predate these estimates that suggest the family divergence in the Jurassic era before breakup of the supercontinent Pangea and two major Asian lineages in early Cretaceous (Dorji et al., 2025a Chapter 3). However, like any predecessor Rhaphidophoridae phylogenetic studies, these conclusions are limited by the lack of reliable ingroup fossils and small sample size representing the lineages though I incorporate mitogenomic data set for our calibrations. Accessing samples and generating genetic data from this region remain major challenge in our understanding of evolutionary history of wingless orthoptera in south and southeast Asia.

Phylogeography provides a powerful lens for examining how genetic lineages are distributed across landscapes. Whether applied to fragmented habitats or elevation gradients, it can reveal how population structure and species distributions are shaped by dispersal limits, and evolutionary divergence. In the future I hope to focus on the steep elevation gradient that characterises Bhutan (from tropical lowland to alpine 4500 m asl) but until species distributions are mapped this is not possible. In contrast, caves create isolated ecosystems, which are also suitable system for addressing phylogeographic questions. Troglophilic camel crickets (hypogean) due to their high degree of dependency on cave habitats and poor dispersibility, the extent of geneflow between population is expected low compared to other epigean species. The narrow geographic range that is typical of troglomorphic populations, along with their high level of endemism, usually cause distinct biogeographic patterns. On the other hand, troglonemes (epigean) species often have more complex patterns due to their high dispersal ability, recolonizations, and recurrent episodes of extinctions (Sbordoni et al., 2000). In recent years several facets of phylogeographic studies of cavernicolous camel crickets, distributed across different region of world, have attempted to relate spatial molecular structure as outcome of to various historical intrinsic and extrinsic processes such as (1) underground karst connection (Caccone, 1985; Caccone & Sbordoni, 1987), (2) surrounding environment of caves (Allegrucci et al., 1997; Cesaroni et al., 1997; Sbordoni et al., 1991), (3) geomorphology and surface structure (Hutchison et al., 2016), (4) ecological behaviours/adaptation (Caccone, 1985; Di Russo et al., 1994), and (5) dispersal capabilities of camel crickets (Johnson et al., 2007; Weckstein et al., 2016). My study expands our understanding of these phylogeographic hypotheses by assessing the degree of connectivity among cave populations by testing whether these cave samples carry genetic signals indicating they function as discrete or as continuous population pattern (Chapter 4). Extensive future sampling across multiple loci and populations has the potential to increase our understanding of the evolutionary role of ecological,

environmental and geographical processes in determining distributional patterns, and impact of rapidly changing temperature and habitat fragmentation on the genetic diversity.

Climate and Climate Change–Future Directions

The steep elevational gradients and the complexity of climatic patterns make mountain ecosystems extremely vulnerable to temperature fluctuations (Klein et al., 2016; Koot et al., 2022). Species distribution models can be used to estimate species geographic ranges in the past, present and future and therefore documenting current species diversity and mapping species ranges should be a conservation priority (Maréchaux et al., 2017; Meza-Joya et al., 2025). As a biodiversity hotspot the Himalaya may be more sensitive to climate change than anywhere outside of the Arctic and Antarctic (Qiu, 2008). When the full geographic range of the newly described raphidophorid species of Bhutan have been documented, I plan to model species distributions to infer past ranges and predict future shifts in their distribution with climate change. In recent years, the Himalaya region has experienced an increase in human-caused activity, resulting in a substantial modification of the environment and a shift in the surface temperature of the region (X. Cui & Graf, 2009). In just a few decades, the Himalaya and Tibetan Plateau have warmed by 1.8° C (Rahim et al., 2023; Yang et al., 2014): a relatively high compared to the northern hemisphere and the world mean temperature (Kang et al., 2010). In addition, the rates of annual and seasonal elevational warming have risen at higher elevations (Liu & Chen, 2000). Such a rate of warming has significant effects on the environment, and species distributions are likely to shift in response (Ingty et al., 2023; Singh et al., 2024; Wambulwa et al., 2021; Dimri et al., 2022)

Mountains are recognized as the primary geographical area to investigate early warning signs of climate change (Liu & Chen, 2000; Koot et al. 2022). In recent decades, climate change impact, exacerbated by anthropogenic activities have increased in many mountain regions, with observable and serious consequences on ecosystems (Meza-Joya et al., 2023). Some changes include loss of glacier mass, rapid permafrost thawing, increase in number and size of glacier lakes and associated geohazards risk in the region. Tibetan plateau and surrounding regions alone have around 97,760 glaciers with an area of about 1000,000 km² (Yao et al., 2012), the third largest outside the polar regions. In past half-century, almost 82% of glaciers have retreat (Qiu, 2008), and this trend has intensified during the past ten years (Sun et al., 2018; Yao et al., 2007, 2012). The growing number of glacial lakes that feed other lakes and the size of the area that they cover raise considerable concerns about the possibility of future geohazards (Li et al.,

2021). Permafrost is another aspect of mountain cryosphere severely impacted by 0.2–0.5°C temperature rise from 1970s to 1990s in Tibetan Plateau, which potentially caused 100,000 km² reduction in permafrost size over the same period (Kang et al., 2010). Accelerated air aerosol thickness and black carbon emissions in the region have accelerated temperature rise (Lau et al. 2010), resulting to fast snow melting (Ballesteros-Cánovas et al., 2018). This has caused climatic variability across west-east and north-south gradients, such as colder to drier and wetter to warmer conditions (Bajracharya et al., 2015). Increasing the risk of catastrophic snow avalanches, flash floods, landslides, and erosions (Cui & Jia, 2015), causing significant natural and socioeconomic losses (Stäubli et al., 2018).

Human-caused climate change is a dynamic force in the 21st century, with far-reaching social, economic, and biological consequences. Over the past 130 years, the global average temperature has increased by approximately 0.85°C and is projected to continue to rise at a rapid rate (IPCC, 2022). Several scientific findings have demonstrated many aspects of anthropogenic-induced climate change will negatively affect biological diversity. The prominent and frequently studied impacts include local population loss (Malcolm et al., 2006; Sekercioglu et al., 2008; Sinervo et al., 2010), habitat displacement (Freeman et al., 2018; Ramirez-Villegas et al., 2014; Roth et al., 2014), and mass mortality events of plants (Smale & Wernberg, 2013; van Mantgem et al., 2009) and animals (Conradie et al., 2020; Ratnayake et al., 2019), resulting in the first climate driven extinctions. According to the IPCC's sixth assessment in 2022, global temperature is expected to rise between 1.5 and 3 degrees Celsius by the year 2100. In such a global climate scenario, many species and ecosystems will be subjected to temperatures exceeding their historical experience, and responses may be prominent in biodiversity hot spots (Trew & Maclean, 2021).

Globally, anthropogenic climate change poses the greatest threat to organisms and their ecosystems (Harvey et al., 2023; Thomas et al., 2004). Many studies have highlighted concerns that climate change might be one of the most serious threats to global biodiversity. The apparent reaction of species to climate change is shift in their range to higher elevation. But does mountain diversity adapted to particular elevation range have a time to adapt to this rapid change? Even though some species exhibit a degree of niche adaptability, many are believed to have irreversible adaptation and are considered to have a limited ability to cope with large variations in their surrounding environment. Increasing pressures due to climate change are anticipated to increase the number of potentially impacted species, resulting in diverse and

unpredictable alterations in community structure, where specialized species will experience drastic reductions in their ranges (or even extinction), and generalist species will spread into new available habitats.

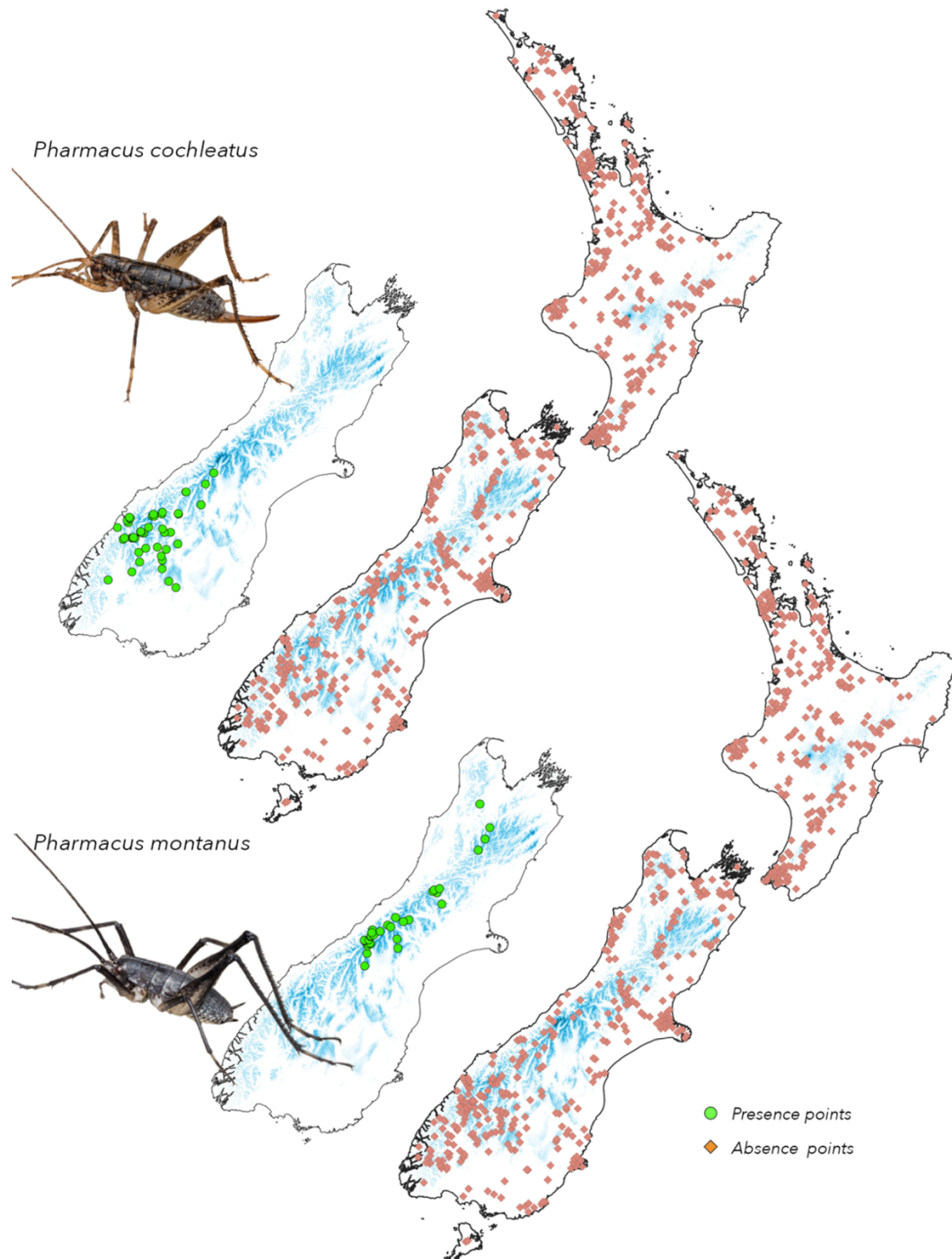


Figure 1. Presence and absence locality maps of two New Zealand Rhaphidophoridae *Pharmacus cochleatus* (top) and *P. montanus* (bottom) based on a total of 40 and 30 presence points were retrieved from various sources both published and unpublished literature (Table S1 and S2) and 800 absence data from iNaturalist respectively. *Pharmacus cochleatus* and *P. montanus* are considered as alpine specialist and tolerance to freeze. Their current distribution

is restricted above tree lines in permanent snow and share similar habitat along the central mountains of South Island (see more details Hegg et al., 2022).



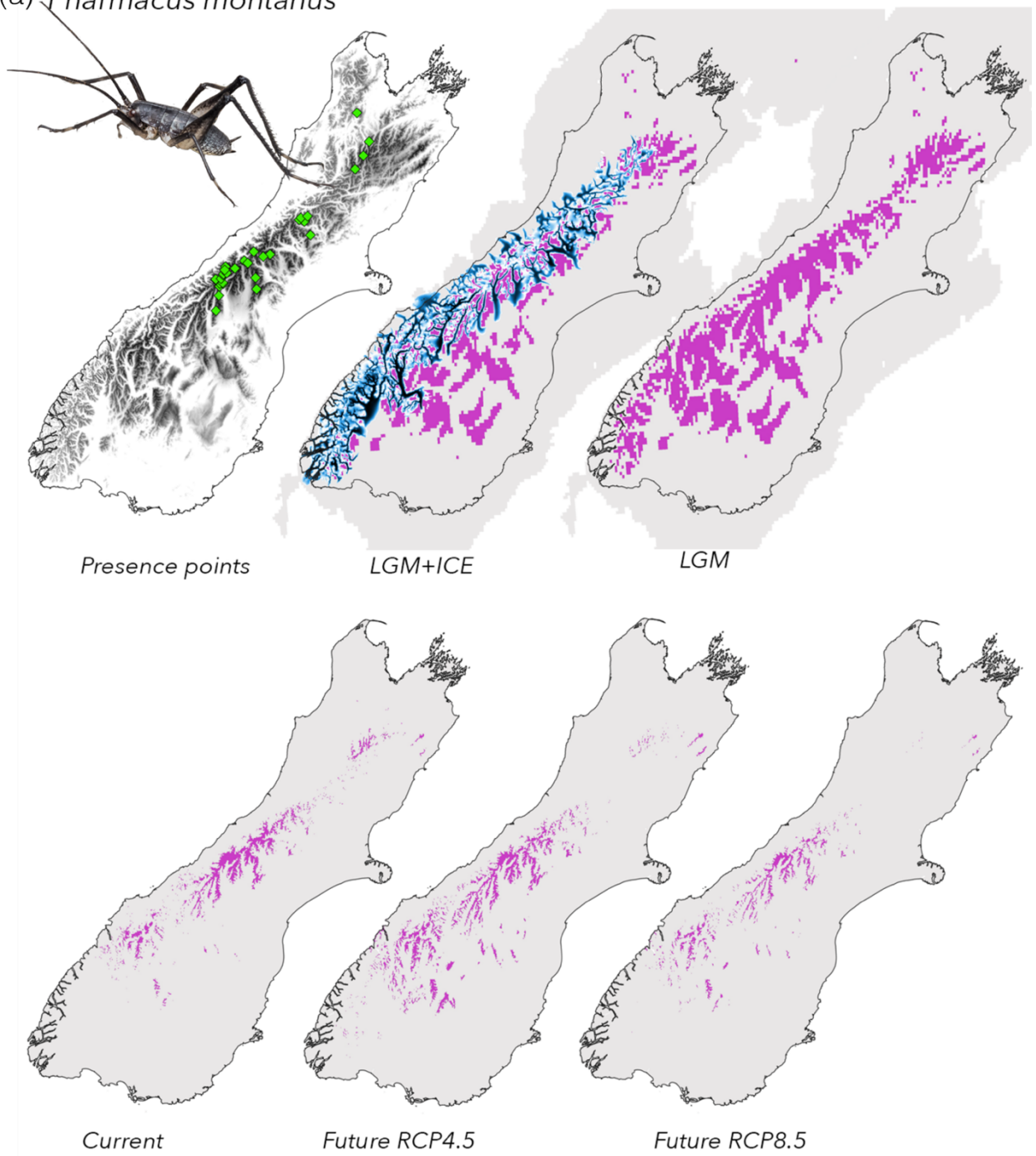
Figure 2. New Zealand alpine Rhaphidophoridae. a–b. *Pharmacus montanus* “Mount Cook Flea”; c–d. *Pharmacus cochleatus*. Photo courtesy Hegg et al., 2022.

Here I provide examples of how species distribution models can be used for alpine Rhaphidophoridae from New Zealand (Fig. 1, 2 and 3). Studies of alpine orthoptera from New Zealand have already identified the threat of population fragmentation and genetic erosion from climate change (Meza-Joya et al., 2023; Koot et al., 2022; Bertoia, 2023) but these models are for diurnal species. Flightless nocturnal alpine Rhaphidophoridae such as *Pharmacus* (Fig. 2) are also at risk (Hegg et al., 2022). In the context of climate change, mountains are the most extensive and significant topographic region. The organism inhabiting mountain alpine are predicted to be particularly vulnerable to the ecological impacts of climate change (Koot et al., 2022; Meza-Joya et al., 2025). High sensitivity to climatic variation and a decreasing amount of suitable surface area as elevation increases limit the ability of alpine adapted organisms to shift their ranges. The habitat for this group of organisms highly constrains to small patch of areas separated by large extend of inhabitable mountain valleys and rivers. Fragmentation and patch size reduction, when coupled with poor dispersal ability, will undoubtedly negatively

affect the ability of species to track and/or shift their range under future climatic change pressure.

Applied at the species level (Fig. 3), species distribution models can help expand our understanding of the climatic forces in shaping intraspecific variability and provide important insights into the factors determining biotic responses to environmental changes. Historic climatic events, particularly the glacial and interglacial periods of the Quaternary climates, have acted as major drivers of speciation, extinction, and diversity, causing significant biogeographic restructuring and affecting evolutionary trajectories (Koot et al., 2022; Meza-Joya et al., 2024; Stewart et al., 2009). The spatial patterns of species distributions thus reflect both past and present environmental changes, corroborated not only by climatic variability but also by abiotic and biotic stressors such as latitude and topography, ecological adaptation and dispersal ability (Hewitt, 2004; Stewart et al., 2009; Đurović et al., 2021). Understanding these mechanistic drivers is crucial, especially for peripheral populations (e.g., alpine species) where exposure to severe and unpredictable stressors may limit adaptation or lead to extinction, as hypothesized by the centre-periphery hypothesis (Camps et al., 2021; Pironon et al., 2017). The interaction between climate, landscape, and species traits determines patterns of biodiversity and evolutionary responses, making it essential to integrate climatic and ecological data to infer the complex phylogeographic dynamics of species range and adaptation.

(a) *Pharmacus montanus*



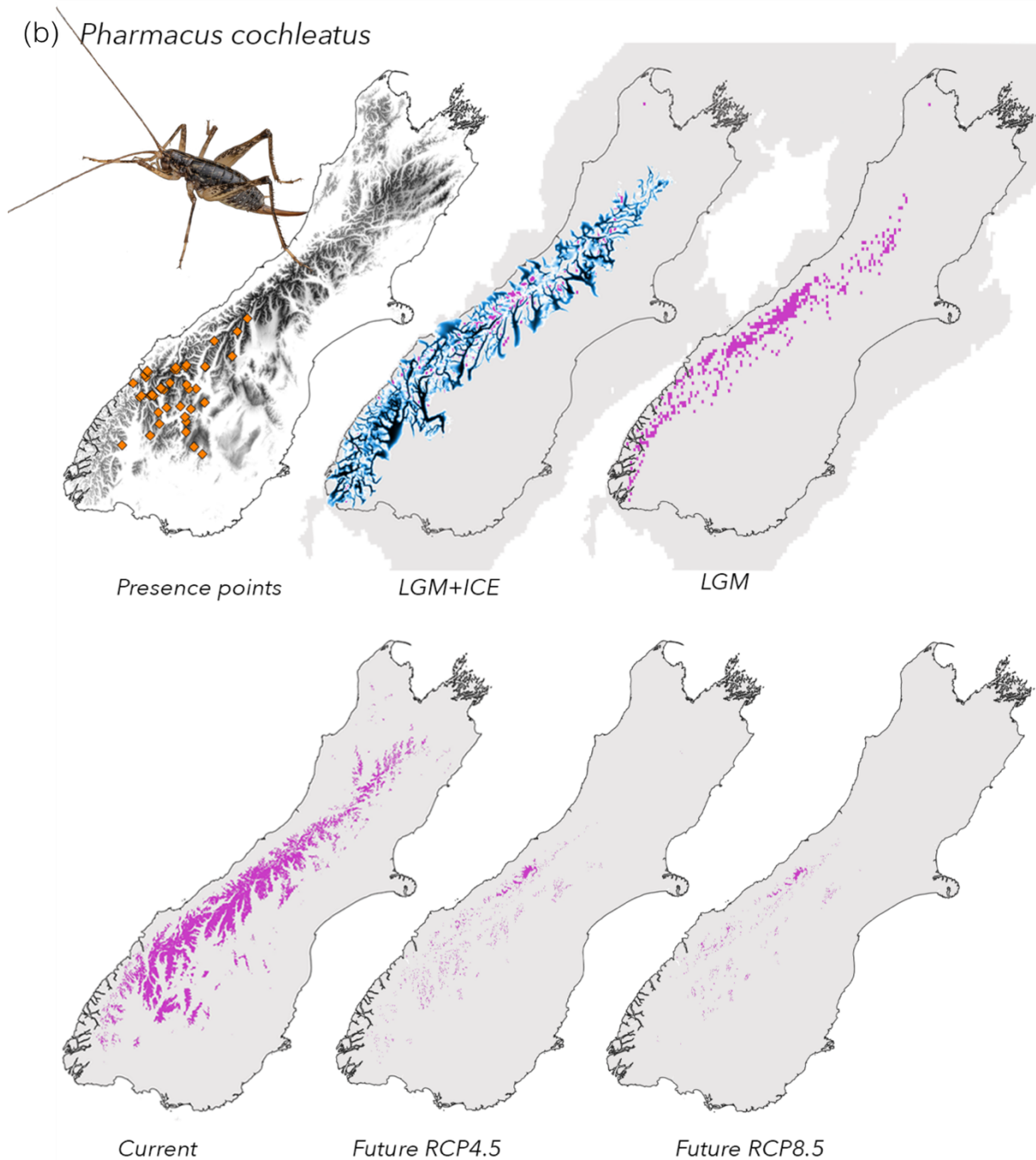


Figure 3. New Zealand *Pharmacus* (Hegg et al., 2022) raphidophorids as an example of species distribution modelling using climate data to infer optimal habitat range and fragmentation in current conditions. Hindcasting allows hypotheses about the influence of past conditions to be developed that can potentially be tested using population genetic approach. Forecasting provides a framework for predicting the trajectory and outcome of ongoing, rapid, anthropogenic climate change. Niche inferred by ecological niche modelling R package BIOMOD 2 using past (Last Glacial Maximum) and two future climate envelopes (RCP4.5 and RCP8.5) for (a) *Pharmacus cochleatus* and (b) *Pharmacus montanus*. Modelling under current climatic conditions, *P. cochleatus* is found to have more suitable habitats than *P. montanus* and opposite scenario under the LGM models. These clearly indicate that they occupied large extend of same habitat and were later separated by some geological and

climatic events. Overlay of LGM extent of glaciation (James et al., 2019) indicates *Pharmacus* may have occupied nunataks during ice ages. In particular, the *P. montanus* model shows inferred habitat among glaciers (on ridges) but also the existence during the LGM of more extensive areas of potentially suitable habitat. The habitat of *P. cochleatus* appears to have been strongly restricted to elevated areas of the Southern Alps amidst intense glaciations. The future projection under both the global climate projection shows an extensive reduction in suitable habitats for both species, pushing them to the higher limits of mountains and to small habitable habitats on southern edge of the South Island.

In summary, this thesis examined one element in the rich invertebrate fauna of Bhutan to provide a framework for understanding the origins and patterns of species diversity but has just scratched the surface. I discovered and described new Rhaphidophoridae species from Eastern Bhutan but diversity in the Himalayan region is undoubtedly much higher. The two subfamilies, Rhaphidophorinae and Aemodogryllinae, that overlap in Asia potentially diverged through a series of dispersal events from Gondwana to Southeast Asia, rather than from walking over the Bering land bridge as previously hypothesized. My examination of genetic differentiation among populations of cave associated camel crickets provided insights into the phylogeography of these global taxa. Future work will incorporate species distribution models to predict how climate will shift geographic ranges of these neglected insects in the biodiversity hotspot of the Himalaya.



CHAPTER 5 | THESIS DISCUSSION

ཡོན་ཏན་དཔེ་ལོ་སྒྲིག་བྱེད་པ་ལ། ཞེས་བཞིན་མཉེན་ཏུ་མས་པར་སྒྲ། རྒྱ་བྲིས་བསམ་གསལ་པས་འགྲེངས་བྱེད་པར་ཡང་། ཐོ་ན་ཅི་ལ་ཕྱི་ཏུ་འཛད།
རྒྱ་དཔེ་ཞིང་གི་བསྐྱེད་བཅོས།

Education is a lifelong journey that can be difficult at first however, it is easily forgotten if not practiced regularly.

Just as filling a pot drop by drop takes effort, all can be lost quickly if not maintained with care.

From the Sutra of water and tree.



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CHAPTER 5 | THESIS DISCUSSION

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SUPPLEMENTARY INFORMATION

Ecological Niche Modelling (Methods and Materials)

Two endemic alpine species of *Pharmacus*; *P. cochleatus* (Karny, 1935) and *P. montanus* Pictet & Saussure, 1891 restricted to South Island mountains were studied. A total of 40 and 30 presence points were retrieved from various sources both published and unpublished literature (Table S1 and S2). The points of old localities from Richards 1972 were retrieved to its approximate localities using New Zealand Topograph and QGIS. The points were checked for duplicates, and additional points within a 1 km² pixel. The presence data of all the New Zealand cave weta available in iNaturalist were treated as absence data for both species. Out of 3500 absence localities, only 800 was used for analysis after removing duplicates and additional points within 3 km². These location points were used to produce species distribution map using QGIS version 3.33.9 Białowieża. This provided comprehensive overview distribution maps of two species in the alpine mountains, and ensured no outliers were included (Fig. 1). After having established the distribution of each species, the same locations data was used alongside bioclimatic variables to model ecological niche using R package BIOMOD2 version 3.5.1 (Thuiller et al., 2016). Three different statistical modelling methods: generalized boosting model/ boosted regression tree (GBM), random forest (RF) and maximum entropy (MAXENT) were applied to the 5 environmental variables and presence/absence data of each *Pharmacus* species. The models were calibrated using 80% of the input data, with the remaining 20% utilised to test them. Variable importance was evaluated by setting the prevalence value to 3. Then generated models for each species were evaluated using receiver operating characteristic (ROC) and true skill statistic (TSS). Only the models with ROC and TSS values of 0.91 and above were considered for building ensemble model. The projection was applied for two future climate and past scenarios.

Table S1. List of presence localities of *P. cochleatus* (including four subspecies) after removing duplicates and thinning to one point at 1 km pixel (Hegg et al., 2022).

Scientific name	Latitude	Longitude	Place
<i>P. cochleatus</i>	-45.5710	169.1053	Tooth Peak, Wakatipu
	-45.4678	168.9529	Treble Cone 9381
<i>P. cochleatus cochleatus</i>	-45.3893	167.5915	Major Peak, Wakatipu
	-45.2853	168.1357	Major Peak, Wakatipu
	-45.2642	168.8156	Major Peak, Wakatipu
	-45.1357	168.3213	Mt Edgar Thomson, Aoraki/Mt Cook National Park
	-45.1354	168.3212	Mount Brewster
	-45.1277	168.8294	Mount Brewster
	-45.0617	168.8112	Mount Pisa
	-44.9826	168.3148	Mount Pisa
	-44.9608	168.9306	Coronet Peak, Wakatipu
	-44.9249	168.4748	Coronet Peak, Wakatipu
	-44.9179	168.7344	Earnslaw Burn, Southern Alps
	-44.8828	169.1950	Roy's Peak, Wanaka, Otago
	-44.7639	167.9850	Sentinel Peak, Lake Hawea, Otago
	-44.7583	168.2483	Bevan Col, Mt Aspiring National Park
	-44.7504	168.1727	Bevan Col, Mt Aspiring National Park
	-44.7459	168.2045	Bevan Col, Mt Aspiring National Park
	-44.7452	168.0165	Turret Head, Earnslaw Burn
	-44.7436	168.2174	Turret Head, Earnslaw Burn
	-44.6934	169.0463	Matukituki River East Branch / Albert Burn
	-44.6924	168.9099	Albertburn Saddle, Otago
	-44.6851	168.4426	French Ridge, Mount Aspiring NP
	-44.6747	168.3934	Ahuriri River East Branch, Canterbury
	-44.6576	168.3798	Ahuriri River East Branch, Canterbury
<i>P. cochleatus fiordensis</i>	-44.6568	168.3865	Emily Peak, Ailsa Mountains
	-44.6335	168.8716	Black Lake, Gertrude Valley, Fiordland National Park
	-44.5963	168.5640	Pt 1952m, Humboldt Mountains
	-44.5713	167.8743	Pt 1952m, Humboldt Mountains
	-44.5046	168.1209	Mt Luxmore, Fiordland National Park

Table S1. (cont.).

Scientific name	Latitude	Longitude	Place
<i>P. cochleatus nauclerus</i>	-44.4682	168.1009	Pt 1552m, Skippers Range, Fiordland National Park
	-44.4391	168.1652	Pt 1290m, Skippers Range, Fiordland National Park
	-44.4351	168.1704	Pt 1290m, Skippers Range, Fiordland National Park
	-44.4252	168.6987	Pt 1290m, Skippers Range, Fiordland National Park
	-44.4201	168.1540	Pt 1423m, Skippers Range, Fiordland National Park
	-44.4106	169.2401	Skippers Range High Point, Fiordland National Park
<i>P. cochleatus rawhiti</i>	-44.4024	169.2350	James Peak, Hector Mountains
	-44.3987	168.8750	James Peak, Hector Mountains
	-44.3975	168.8731	Gem Lake, Umbrella Mountains
	-44.3942	168.6876	Blue Lake Creek, Garvie Mountains
	-44.3699	168.8887	Blue Lake Creek, Garvie Mountains
	-44.2804	169.7476	Mt Cerberus, Mavora, Southland
	-44.0662	169.4238	Mount Turnbull, Thomson Mountains
	-44.0655	169.4284	Mount Turnbull, Thomson Mountains
	-43.9524	169.8624	Lake Alta, The Remarkables, Otago
	-43.7825	170.0556	Te Anau Downs

Table S2. List of presence localities of *P. montanus* after removing duplicates and thinning one point at 1 km pixel (still updating).

Scientific name	Latitude	Longitude	Place	Source
<i>P. montanus</i>	-43.8712	170.0454	Mount Dark, Ben Ohau Range	Hegg et al., 2022
	-43.7513	170.0621	Mount Annette, Aoraki / Mount Cook National Park	
	-43.7199	170.1277	Mount Wakefield, Aoraki/Mount Cook Natinal Park	
	-43.7150	170.0698	Sealy Range, Aoraki/Mount Cook National Park	
	-43.7071	170.1217	Mount Wakefield, Aoraki/Mt Cook National Park	
	-43.6667	170.0003	Westland District, West Coast	
	-43.6591	169.9992	Bluewater Saddle, Sierra Range, West Coast	
	-43.6567	170.7081	Timaru, Mesopotamia, Canterbury	
	-43.6537	170.0948	7999, New Zealand	
	-43.3190	170.6901	Whataroa 7886	
	-42.9436	171.5912	Mt O'Malley, Arthur's Pass National Park, Canterbury	
	-42.9200	171.5026	Selwyn District, Canterbury	
	-42.8959	171.5338	Otira Valley, Arthurs Pass	
	-42.8757	171.6505	Lake Anna, Mt Franklin, Arthur's Pass NP	
	-42.2744	172.4900	Gloriana Peak, Spenser Mountains	
	-42.2744	172.4899	Gloriana Peak, Spenser Mountains	
	-41.9193	172.7352	Travers Range, Nelson Lakes NP	
	-41.5471	172.5286	Poverty Basin, Mt Owen, Kahurangi National Park	
	-43.594819	170.14226	Canterbury, Mt. Cook region	
	-42.102	172.635	Mt Mahanga, Spenser Mountains, Nelson	Richards, 1972
	-43.35996	170.97626	Piton Peak, Arrowsmith Range, Canterbury	
	-43.40232	170.85889	Jollie Range, Canterbury	
	-43.83183	170.67756	Two Thumb Range, Canterbury	
	-43.58037	170.19404	Haast Ridge, Mt Cook, Canterbury	
	-43.50712	170.18801	Summit Mt Van Bulow, Fritz Range, Westland	
	-43.19425	171.63209	Remarkable Ridge, Craigieburn Range, Canterbury	
	-44.064	169.98409	Ben Ohau Range, Canterbury	
	-43.51671	170.11546	Fox Glacier, Westland	
	-43.48036	170.51042	Godley Valley, Canterbury	
	-43.46491	170.58167	Mt D'Archiac, Godley Valley, Canterbury	

Table S3. Environmental predictor variables initially investigated for ecological niche modelling. Soil data from the New Zealand Land Resource Information System portal (<https://iris.scinfo.org.nz>) was simplified from 72 to 20 major classes. Variable selection was carried out using the multGLM function in the R package ‘fuzzySim’ v.3.0 (Barbosa, 2015) which implements a stepwise selection procedure considering correlations among variables, false discovery rate, and parsimony. Variables retained for final modelling depicting their final Variance Inflation Factor (VIF) value. Variable type is shown (dyn = dynamic, sta = static, con = continuous, cat = categorical).

Code	Description	Variable	<i>P. cochleatus</i>	<i>P. montanus</i>
bio1	Annual mean temperature (°C)	dyn, con	-	-
bio2	Annual mean diurnal range (°C)	dyn, con	-	-
bio3	Isothermality (%)	dyn, con	-	-
bio4	Temperature seasonality (SD, °C)	dyn, con	-	-
bio5	Max temperature of warmest month (°C)	dyn, con	-	-
bio6	Min temperature of coldest month (°C)	dyn, con	-	-
bio7	Temperature annual range (°C)	dyn, con	-	-
bio8	Mean temperature of wettest quarter (°C)	dyn, con	-	-
bio9	Mean temperature of driest quarter (°C)	dyn, con	-	-
bio10	Mean temperature of warmest quarter (°C)	dyn, con	-	-
bio11	Mean temperature of coldest quarter (°C)	dyn, con	1.452	1.307
bio12	Annual precipitation (mm)	dyn, con	-	-
bio13	Precipitation of wettest month (mm)	dyn, con	-	-
bio14	Precipitation of driest month (mm)	dyn, con	-	-
bio15	Precipitation seasonality (CV, %)	dyn, con	-	-
bio16	Precipitation of wettest quarter (mm)	dyn, con	-	-
bio17	Precipitation of driest quarter (mm)	dyn, con	-	-
bio18	Precipitation of warmest quarter (mm)	dyn, con	1.830	2.593
bio19	Precipitation of coldest quarter (mm)	dyn, con	1.142	2.153
slope	Slope (°)	sta, con	-	-
asp	Aspect (°)	sta, con	-	-
rough	Roughness (°)	sta, con	-	-
soil	Soil type (class)	sta, cat	-	-

Table S4. List of tuneable hyperparameter tested using the R package ‘SDMtune’ v.1.1.4, indicating the best performing parameters used for refitting the final models for each species. Default values as implemented in the R package ‘Biomod2’ v.3.4.6. The meaning of each tuneable hyperparameter can be found in the respective package documentation. Feature classes for Maximum Entropy are (l) linear, (q) quadratic, (p) product, and (h) hinge.

Algorithm	Tuneable options	Default	Settings tested	Best performing parameters	
				<i>Pharmacus cochleatus</i>	<i>Pharmacus montanus</i>
Generalised Boosting Model	number of trees	2500	750, 1250, 2500, 5000	750	1250
	interaction depth	7	1, 3, 7, 10	10	7
	shrinkage	0.001	0.0005, 0.001, 0.002, 0.004	0.0005	0.001
	bag fraction	0.50	0.25, 0.50, 0.75, 1	0.5	0.5
Random Forest	# Sampled variables	sqrt(#var)	1, 1.5, 2, 2.5, 3, 3.5, 4	3	2
	number of trees	500	250, 500, 1000, 2000	1000	500
	size of terminal nodes	5	1, 5, 10, 15, 20	15	5
Maximum Entropy	feature classes	lqpht	l, lq, lh, lqp, lqph, lqpht	lqph	lqpht
	Regularisation multiplier	1	0.1, 0.5, 1, 1.5, 2, 2.5, 3	1.5	1

Table S5. The final mean-weighted ensemble models (EMmw) showed a high performance as indicated by their receiver operating characteristic (AUC), sensitivity, and specificity values. Cut-off values for generating binary maps are shown.

Species	AUC	Cut-off	Sensitivity	Specificity
<i>Pharmacus cochleatus</i>	0.991	441	100	96.28
<i>Pharmacus montanus</i>	0.995	263	100	97.80

Table S6. Temperature related predictors had the most significant influence on the environmental envelope estimated for both species (top: *P. cochleatus* and bottom: *P. montanus*). Variable importance scores and confidence bars (95%) are reported as percentages of total probability scores for the final mean-weighted ensemble models (EMmw). Variable codes as in Table S2.

Variables	GBM	RF	MAXENT.Phillips
bio11	0.7136	0.6724	0.8346
bio18	0.478	0.4628	0.4342
bio19	0.0406	0.128	0.047

Variables	GBM	RF	MAXENT.Phillips
bio11	0.8286	0.659	0.8846
bio19	0.3756	0.093	0.197
bio18	0.0162	0.05	0.1396



