The role of community science in orthopteran research

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Abstract

Orthopterans are commonly encountered in rural, suburban, and urban landscapes and have charismatic songs that attract the public's attention. These are ideal organisms for connecting the public with science and critical concepts in ecology and evolution, such as habitat conservation and climate change. In this review, we provide an overview of community science and review community science in orthopterans. Best practices for orthopteran community science are provided, with a focus on audio recordings and highlighting new ways in which scientists who study orthopterans can engage in community science.

Before the modern era, scientific discovery was commonly made by people who were not scientists by profession (Brenna 2011, Miller-Rushing et al. 2012). This began to change in the middle of the nineteenth century when science became highly academic, with greater "gatekeeping" of knowledge, and data collection became increasingly expensive. As a result, much of the knowledge gained during that time has been effectively withheld from non-scientists in difficult-to-obtain scientific journals, and there were few opportunities for the public to directly engage with scientific research. In recent years, there has been a concerted effort from the scientific community to change the way we engage with the public. These "citizen" or "community" science projects are filling gaps in the modern approach to scientific inquiry (Jordan et al. 2012, Toomey and Domroese 2013, Johnson et al. 2014). Here, we provide an overview of community science and highlight the exciting and unique role that community science can play in orthopteran research. We focus on how acoustic surveys can be used to study orthopteran biodiversity, provide best practices for orthopteran community science, and suggest future avenues for research.

Keywords

acoustic monitoring, best practices, citizen science, community science, crickets, grasshoppers

The importance of community science

Community science refers to the participation of people who are not professional scientists in scientific inquiry through the collection, analysis, and interpretation of scientific data (Jordan et al. 2012, Toomey and Domroese 2013, Johnson et al. 2014). There are typically two main avenues for community science, which we will refer to as "guided" and "open." In guided community science studies, scientists lead the data collection, usually using an established protocol, with varying degrees of input from local volunteers and organizations. In these studies, community scientists work directly with researchers or in tandem with them on web platforms such as Zooniverse (https://www.zooniverse.org/). In open community science studies, data are generated largely by individuals working independently and are then recorded and shared through social media or apps such as iNaturalist (https://www.inaturalist.org/; Paiero et al. 2020, Skejo et al. 2020b, Kasalo et al. 2021a, 2021b, Trewick 2021). These internet-based forums provide anyone with a smartphone or computer the ability to add to a collective database that is accessible by scientists and nonscientists everywhere.

Community science is changing the way scientists can collect data, increasing both their resources and reach (Silvertown 2009, Jordan et al. 2015). Although community science initiatives usually provide fine-scale data at a local level, they can cover large regions collectively (Theobald et al. 2015). This allows community science projects to gather much more data than a small group of scientists would alone (Pocock et al. 2015, Kaláb et al. 2021). For example, organized initiatives led by passionate amateur scientists are valuable in tracking changes in populations over time (Pocock et al. 2015). Locals have the ability to record data year-round, which would be

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difficult and costly for scientists who are based far from their study sites (Kaláb et al. 2021). Moreover, local knowledge of an area can be invaluable to scientists conducting fieldwork (Penone et al. 2013, Medin and Bang 2014). The geographic scale and depth of community science surveys are particularly valuable in the context of anthropogenic change-the scale and speed at which humans are impacting biodiversity require the collection of as much data as possible as quickly as possible (Theobald et al. 2015). Community science initiatives have been successful in monitoring conservation efforts (Barlow et al. 2015, Kallimanis et al. 2017), sighting species thought to be extinct (Woller and Hill 2015, Buzzetti et al. 2021), discovering new species (Kasalo et al. 2021b, Trewick 2021), locating occurrences of range expansion (Beckmann 2017, Paiero et al. 2020, Kaláb et al. 2021), and invasive species (Okayasu et al. 2020, Ahnelt et al. 2021, Kasalo et al. 2021a). In some taxa, most newly discovered species are first described by people who are not professional scientists (Fontaine et al. 2021).

Community science is equally important for promoting public engagement with science. Community science provides people with a way to have meaningful scientific experiences that translate into significant and lasting learning. Moreover, community science makes the scientific experience more accessible to members of historically marginalized groups (Skejo et al. 2020b) and in underserved classrooms (Fiske et al. 2019, Roche et al. 2020). A focus on justice, equity, diversity, and inclusion in community science can also bring added value to the research. For example, involving indigenous peoples in research based on their native lands brings immense value to the quality of the research through the provision of differing perspectives and contexts (Kimmerer 2002, 2013) and to the consideration and preservation of indigenous cultures (Medin and Bang 2014).

Community science in orthopteran research

Orthopterans are familiar occupants of rural and suburban backyards as well as urban parks and vacant lots, providing an acoustic backdrop to summer. These are the ideal organisms to connect the public to science and to critical concepts in ecology and evolution, such as habitat conservation and climate change. Insects make up one of the largest shares of the Earth's biodiversity, but recent reports on severe insect declines are alarming (Sánchez-Bayo and Wyckhuys 2019). Because of their short life cycles and, in some species, specialization in habitat, food source, and egg-laying, insects are excellent indicators of climate change (Riede 1998, Jeliazkov et al. 2016, Beckmann 2017). For most insects, we still have too little information about extant biodiversity to understand the causes and consequences of population declines (Saunders et al. 2019). Due to their ubiquity and sensitivity to climate change, orthopterans are particularly important organisms for climate change research (Fartmann et al. 2012, Löffler et al. 2019). Continuous monitoring through organized citizen science can contribute to long-term datasets that will help to track changes in biodiversity, while providing the public with meaningful science experiences (Basset and Lamarre 2019). Currently, there are 1,128,486 records on iNaturalist that are accompanied by photographs and observation localities for 5,732 orthopteran species (iNaturalist, available from https://www.inaturalist.org. Accessed July 14, 2022).

Because many male orthopterans sing to attract mates, community science studies quantifying species richness, abundance, and emergence times in Orthoptera are relatively simple. Species can be identified by their acoustic profiles, and acoustic survey data can be recorded from trails and roadsides (Fischer et al. 1997, Riede 1998, Penone et al. 2013, Jeliazkov et al. 2016, McNeil and Grozinger 2020, Paiero et al. 2020, Kaláb et al. 2021). This is particularly useful in fragile habitats or for threatened species, where scientists must balance effective monitoring with reducing disruption in conservation spaces (Moran et al. 2014, McNeil and Grozinger 2020). New technologies in acoustic monitoring allow for large-scale monitoring of singing insects, which provides an easier, less time-consuming means of estimating metrics such as species abundance and richness. Community scientists can sustainably crowdsource this vital information in a way that scientists are not able to do using a traditional approach or photographs alone.

Nearly 85% of the world's population owns a smartphone (Turner 2018). Every smartphone has audio and video recording, GPS, and internet capabilities, placing these tools for data collection, storage, and transmission at the fingertips of most people on the planet. Highly accurate new tools, such as TADARIDA (a Toolbox for Animal Detection in Acoustic Recordings Integrating Discriminant Analysis) and AI, make using the vast quantities of acoustic and photographic data generated by community scientists useful on a massive scale (Bas 2016, Kasalo et al. 2021b). In the case of acoustic monitoring, data for many different species across taxa can be captured and analyzed from a single recording, a practice that could further utilize existing recordings, increase the rate of new data collection, decrease costs, and encourage collaboration (Jeliazkov et al. 2016, Newson et al. 2017). Smartphone technology also allows us to easily record data that is outside the normal human sensory range, which provides a means to detect species that might otherwise go unnoticed (Moran et al. 2014). Community science acoustic monitoring is currently being used at a nationwide scale in some locations and taxa (e.g., FrogID (Rowley et al. 2019, Rowley and Callaghan 2020); North American Breeding Bird Survey, USGS Patuxent Wildlife Research Center and Environment Canada's Canadian Wildlife Survey).

We reviewed 14 studies that used community science in orthopteran research (Table 1) and found examples of both guided (43%) and open (50%) community science, with the remaining 7% unclear. Research spanned orthopteran taxa with most major groups being represented, including grasshoppers, crickets, katydids, and wetas; however, taxonomic diversity within each of those groups is relatively limited to new or invasive species (Table 1). For guided studies, the number of participants was small, with groups of less than 15 people. In open community science studies, the number of non-professionals who participated was typically not included. In most studies, participants helped collect photographic and/or acoustic data. Acoustic monitoring orthopteran community science initiatives are still underutilized. Only four of the studies we found used community-collected acoustic data (Penone et al. 2013, Jeliazkov et al. 2016, Newson et al. 2017, Kaláb et al. 2021), while the other 10 primarily used photographs, social media, field collection, or a combination of methods to achieve their aims. All 14 studies we surveyed addressed questions of species richness, species abundance, novel/threatened species identification, range changes/expansion, invasive species, and environmental factors impacting species.

We wanted to highlight one ongoing orthoptera research project that addresses experimental evolution questions using communityanalyzed data. The Cricket Wing (https://www.zooniverse.org/projects/marywestwood/the-cricket-wing, Box 1) uses an online platform to host a large dataset of images that are analyzed by the public. This type of online, large-scale data analysis community science provides an alternative to field collection projects and another exciting avenue to expand research participation and speed up scientific discovery.

Туре	Country Organism M		Organism Number of Involvement type		Question type(s)	Authors	
			participants				
Guided	France	11 species of bush crickets	10	Roadside acoustic data	Species richness;	(Penone et al. 2013,	
		(Tettigoniidae family)	individuals	collection	species abundance;	Jeliazkov et al. 2016)	
					environmental factors		
Guided	Germany	Oak bush-cricket (Meconematinae	~8	Photograph collection; social	Range expansion	(Ahnelt et al. 2021)	
		family)	individuals	media			
Guided	United	Bush Crickets (Tettigoniidae family)	Not reported	Placement of static acoustic	Species richness	(Newson et al. 2017)	
	Kingdom			sensors			
Guided	Japan	Pink-winged grasshopper	Not reported	Field specimen collection	Invasive species	(Okayasu et al. 2020)	
		(Pyrgomorphidae family)					
Guided	United States	Camel crickets (Rhaphidophoridae	Not reported	Photograph collection; specimen	Invasive species	(Epps et al. 2014)	
		family)		collection; social media; survey			
Guided	United States	Grasshopper (Acrididae family)	Not reported	Transcription of field journals	Rare species record	(Woller and Hill 2015)	
Open	Australia	Pygmy grasshoppers (Tetrigidae	8 individuals	Photograph collection; social	Rare species record	(Skejo et al. 2020b)	
		family)		media			
Open	Canada	Red-headed bush cricket and restless	~15	Photograph collection; social	Range expansion	(Paiero et al. 2020)	
		bush cricket (Gryllidae family)	individuals	media			
Open	United	Conocephalus discolor and	2000+	Photograph collection	Range expansion;	(Beckmann 2017)	
	Kingdom	Metrioptera roeselii	people		environmental factors		
Open	United States	Acrididae and Romaleidae families	Not reported	Photograph collection; social	Species richness;	(Harman et al. 2022)	
				media	species abundance		
Open	United States	Japanese burrowing cricket	Not reported	Photograph collection; social	Invasive species;	(Bowles 2018)	
		(Gryllidae family)		media	range expansion		
Open	New Zealand	Ground weta (Anostostomatidae	Not reported	Photograph collection; social	New species	(Trewick 2021)	
		family)		media	identification		
Open	Madagascar	Southern Devils pygmy grasshopper	4 individuals	Photograph collection; social	New species	(Skejo et al. 2020a)	
		(Tetrigidae family)		media	identification		
Not	Czech	Bush crickets (Tettigoniidae family)	Not reported	Photograph and acoustic	Range expansion	(Kaláb et al. 2021)	
reported	Republic			collection; social media			

Table 1. Published research on orthopterans that has included a community science element.

Box 1. Orthopteran Community Science in Action: The Cricket Wing.

The Cricket Wing is an ongoing community science initiative and delves into how noise pollution impacts cricket physiology. Because singing and hearing are essential for cricket, and more broadly, orthopteran reproduction, noise pollution can have negative impacts on these organisms. Very little is currently known about how noise pollution impacts orthopterans, especially with regards to their physiology. Specifically, the research underlying The Cricket Wing aims to understand how traffic noise affects immune and reproductive traits.



To date, the lab group running The Cricket Wing has generated two datasets: (i) 12,304 images of live and dead sperm cells to measure reproductive traits; and (ii) 1917 images of immune cells (hemocytes) to measure immune traits. The Cricket Wing, via the Zooniverse platform, engages participants from the community to count live and dead sperm and hemocytes in their respective images. To control for biases and error, each image is "classified" ten different times by participants before final numbers for each image are recorded. Guides and tutorials are provided to community participants for the different tasks carried out on the site. An open chat forum ("The Cricket Wing Talk") is available for participants, scientists, and developers of the site to troubleshoot issues and discuss the broader science behind the project. Since it launched on May 10, 2022, The Cricket Wing has registered 700 participants who completed a total of 38,497 classifications (37,356 sperm and 1141 hemocyte counts) to date (Accessed July 14, 2022). The Cricket Wing is an excellent, real-time example of how community participants can engage in orthopteran research, as well as in broad evolutionary questions. It uses a guided community science approach and follows many of the best practices that we have outlined in the main text. The Cricket Wing

is a way to engage the community in novel research, educate a broader, non-scientific audience about evolutionary theory, and demonstrate how scientific data collection works. Currently, The Cricket Wing is being extended and utilized in outreach at the high school level. The developers and collaborators also plan to extend the scope to other evolutionary questions, such as rapid adaptation through song analysis and machine learning. The Cricket Wing is led by Dr. Robin Tinghitella's lab group (including Dr. Tinghitella, Dr. Mary Westwood, Gabrielle Welsh, and Sophia Anner) at the University of Denver and Dr. Sarah Reece's lab group (including Dr. Reece and Dr. Aidan O'Donnell) at the University of Edinburgh. To learn more about The Cricket Wing, visit https://www.zooniverse.org/projects/marywestwood/the-cricket-wing.

Best practices for community science in orthoptera research

Despite the opportunities for community science in orthopteran research, there are very few organized, long-term community science programs that focus on these organisms (Burton 2003, Fartmann et al. 2012, Newson et al. 2017, Löffler et al. 2019). With this in mind, we propose some best practices for creating effective community science programs in Orthopteran research. This is not meant to be an exhaustive list but rather a starting point to increase awareness, accuracy, and utility.

1. Develop guided community science projects. In general, we recommend guided studies for most avenues of research. Guided studies have been shown to be better suited for some research questions, such as evaluating species abundance (Penone et al. 2013). We also recommend a guided approach because it can be the best way to actively engage with community scientists and provide a more meaningful research experience.

2. Develop clear and concise protocols. Studies have shown that clear, concise protocols are critical for guided studies (Matteson et al. 2012, Penone et al. 2013). Below, we outline some suggestions for information that should be included in the protocol.

2.1. Plan how community scientists will access study organisms. Locals, naturalists, and professional scientists have concerns regarding the damage that numerous untrained visitors can do to fragile ecosystems (Moran et al. 2014). Community science protocols should account for the frequency and manner in which community scientists will access a research area. Protocols should also include guides for how and where to find the study species.

2.2. Include details of how data should be recorded and stored. For acoustics, this would entail including instructions on how to record sound, recommended recording distance, and length of time of recording. This would also include detailing any and all notes, such as locality information, date and time of observation, and general notes on habitat. A plan would also be included for how data might be backed up or shared in a repository such as Google Drive or Dropbox, website submission, or an app like iNaturalist.

2.3. Use automated processes to record data when possible. Automating data collection using a smartphone app can reduce recording errors. Zilli et al. (2014) designed and deployed a smartphone app that uses acoustic data to identify specific species in real time. When designing apps for use by non-scientists, mimicking the design of existing popular apps (i.e., Shazam) can increase user uptake and engagement (Moran et al. 2014).

3. Provide instructional resources. In guided studies, workshops, online tutorials, fieldnotes, and/or video demonstrations should be used to provide training to volunteers (Barlow et al. 2015). In the case of collecting acoustic data, example audio recordings of the subject specie(s) are helpful to participants. In studies that require volunteers to make identifications, it is helpful to include an "unsure" column to reduce guessing when participants are uncertain (Barlow et al. 2015).

4. Engage with community scientists and the general public. Engaging with community scientists and the general public is of paramount importance when conducting community science initiatives and provides a more meaningful learning experience to the research project. This can be done during and after

community science initiatives and can take the form of websites, discussion forums, organized "walks" to identify species, and public talks in which results are disseminated to community participants in the project. Ultimately, community science is great for collecting and processing large amounts of data, but professional scientists should also keep the goal of contributing to public scientific literacy at the forefront.

5. Provide opportunities for practice. The extent, duration, and mode of participant training all have effects on the quality of community science data (Galloway et al. 2006, Delaney et al. 2008, Fitzpatrick et al. 2009, Jiguet 2009, Schmeller et al. 2009). Conducting practice data collection with groups of participants or tutorials that outline methods for data collection can improve the quality of the data being generated.

6. Build replication into data collection. Error and bias due to variations in observer quality, along with differing approaches to data collection, can impact the validity of community science data and subsequent analysis. Several studies have shown how different approaches to the same community science datasets can vield different results and lead scientists to variable conclusions (Bas 2016, Kasalo et al. 2021b). Specifically with respect to the acoustic monitoring of frogs, researchers have found broad inter-observer variation in species identification and have suggested that this should be controlled for in either the sample design or during data analysis (de Solla et al. 2005, Weir et al. 2005, Lotz and Allen 2007, Pierce and Gutzwiller 2007). To mitigate these biases in studies that use community science data, it may be helpful to collect data based on two or more independent observers. For example, for acoustic surveying, have more than one person survey/cover a specific location/area or, in cases where measurements are being taken via a web platform, have several people measure the same thing to add replication to the measurement.

Plan for sampling bias. Sampling biases due to the 7. temporal and spatial heterogeneity of the data collection can also be issues within community science-generated datasets. Both types of sampling biases can add their own set of issues to downstream analyses, as can trying to correct or account for these biases either before and/or after data collection (Harris and Haskell 2007, Niemuth et al. 2007, Dunn and Weston 2008, Dickinson et al. 2010). Researchers using community science data are recognizing that, like working with laboratory or scientifically generated data, there is a learning curve to working with community science-generated datasets and that issues of bias and error within the data must be addressed in a questionspecific manner. Ultimately, finding and achieving the most appropriate balance between analytical techniques, community science-generated/analyzed datasets, and a given research question is a very active area of research.

Conclusions

Community science projects are quickly increasing in number but are drastically underutilized in scientific literature (Theobald et al. 2015). In Orthoptera, projects using acoustic data recorded by community scientists can help answer questions related to species abundance, species richness, emergence time, and changes in range and distribution due to anthropogenic change (Penone et al. 2013); however we were only able to locate 14 published studies that specifically mentioned the use of community science in their methods and only four of which used acoustic monitoring. Community science is growing in popularity and provides many benefits, including increasing scientific knowledge and engaging the general public, enhancing conservation, and providing much-needed work hours to advance research goals. However, these benefits can be outweighed by damage to fragile ecosystems and threatened wildlife if participants are not properly trained. Thus, it appears that community science, as with the natural world it surveys, requires balance to be sustainable. Because they are easily identified through mating song, Orthoptera species provide excellent study systems for achieving all of these goals from distances that can help protect vulnerable habitats.

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Acrida bara, synomymous with A. sulphuripennis (Orthoptera, Acrididae, Acridinae)

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Abstract

The male holotype of *Acrida bara* Steinmann, 1963 from the Budapest collection (HNHM) was studied and found to be identical to *Acrida sulphuripennis* (Gerstaecker, 1869). Consequently, *Acrida bara syn. nov.* is synonymized with *A. sulphuripennis*.

Keywords

East Africa, taxonomy, Tanzania

Introduction

The genus Acrida Linnaeus, 1758 is a species-rich genus of Acridinae widespread in the Mediterranean region, Africa, and Asia. In Africa, A. turrita (Linnaeus, 1758), A. sulphuripennis, and A. bicolor (Thunberg, 1815) are widely distributed, the latter two belonging to the most common species in East Africa. Acrida species are dwellers of grasslands, preferring disturbed habitats and very common in ruderal vegetation. Acrida sulphuripennis is a pioneer species that colonizes fallow lands and opened-up areas along roadsides. It is found from sea level up to the lower border of the montane forest at 1800 m on Mt. Kilimanjaro (Hemp 2009). A. sulphuripennis often occurs syntopically with A. bicolor. Both species are easily distinguished on the basis of wing color, with a matte yellow color in A. bicolor and vivid yellow to orange color in the hind wings in A. sulphuripennis. Gerstaecker (1869) described A. sulphuripennis from Zanzibar, giving only a very short description. Gerstaecker (1873) published a more detailed description of this species and compared it with A. bicolor. Acrida bara was described by Steinmann (1963) after a single male from Lushoto (Lushato in Steinmann 1963) in the West Usambara Mountains. The holotype is deposited in the Natural History Museum Budapest in Hungary (HNHM). Since its description, no further specimens have been reported.

The male holotype of *A. bara* from the Natural History Museum Budapest was studied and compared with a series of specimens of *A. sulphuripennis* from Zanzibar, the Tanzanian mainland, and some other African countries.

Material and methods

The type of *A. bara* was morphologically compared with specimens of *A. sulphuripennis* from eastern and southern Africa (from the Natural History Museum London, UK). Morphological characters compared include the shape and color of the wings, overall habitus, the shape of the head and the pronotum, and the shape of the last abdominal tergites in males.

Results

Steinmann did not directly compare his *A. bara* with *A. sulphuripennis* or, indeed, with any other species. The only reference to *A. sulphuripennis* in his paper is found in his key to species. He defines it there on the basis of three sets of characters: a) tip of tegmen straight and pointed, b) dorsal process of subgenital plate as large as the apex of that plate, and c) hind wing a matte yellow in color. In his description of *A. bara*, he states that its tegmen tip is parabolic, not straight and pointed, and he describes the dorsal projection of the subgenital plate as being "2.5 times shorter than then the length of the apex of the plate." The photos of the holotype presented in this paper show that neither statement is correct. Steinmann gives no information about the color of the hind wing of *A. bara*—it is likely that he never examined it, as the holotype has folded wings and the hind wing is not visible.

Comparing the holotype with specimens coming from Zanzibar (type locality of *A. sulphuripennis*, Fig. 2C), the East and West Usambaras (the latter the type locality of *A. bara*; Fig. 1D), the vicinity of Kilimanjaro (Fig. 2A, B), and the foothills of the East Usambara Mountains (Fig. 2D), no differences in the morphology of the male apex (compare Fig. 1C and Fig. 2C–E), the shape of the wings, the pronotum (Fig. 1B), or coloration and venation could be detected. Additionally, *A. sulphuripennis* from other African countries, e.g., Zambia or KwaZulu Natal (Fig. 2E), were studied at the Natural History Museum in London, and no differences—besides some variation in size and coloration—could be detected.

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Fig. 1. Male holotype of Acrida bara (A-C) and male specimen of Acrida sulphuripennis (D) photographed near the type locality in the West Usambara Mountains of Tanzania A. Lateral habitus; B. Head and pronotum; C. Lateral view of apex. Scale bars 5 mm.

Discussion

In widespread species, variation in external morphology, coloration, and body size is common (e.g., Ibrahim 1974; Bai et al. 2016; Rosetti and Remis 2018), and if the species are only known from a few widely separated localities, this could well lead to the description of separate taxa. When more specimens are collected described as different taxa but later united under a single taxon,

from intermediate localities, taxa described from widely separated localities often turn out to be identical. In various Oedipodinae, such as Acrotylus Fieber, 1853 or Gastrimargus Saussure, 1884, differences in color or body size were found to be simply variations of populations from different localities (Cigliano et al. 2022).

In the genus Acrida, a number of widespread species were



Fig. 2. Male *Acrida sulphuripennis*. A, B. Habitus (Kilimanjaro) C–D. Lateral view on male apex of specimens from Zanzibar near Jozani (C), from Muhesa at the foothills of the East Usambara Mountains (D), and from KwaZulu Natal (E). Scale bars: 5 mm.

e.g., *A. bicolor* (8 synonyms), *A. cinerea* (Thunberg, 1815) (6 synonyms), *A. exaltata* (Walker, 1859) (4 synonyms), and *A. turrita* (7 synonyms) (Cigliano et al. 2022). Museum collections serve as an indispensable base for understanding the biogeography of species, enabling taxonomists to compare larger series of any given species and to decide on its species status.

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Sexual dimorphism in the badlands cricket (Orthoptera, Gryllinae, Gryllus personatus)

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Abstract

Sexual dimorphism (SD) is a common phenomenon in sexual species and can manifest in a variety of ways. Sexual size dimorphism (SSD) is commonly investigated, but it can be confounded with sexual shape dimorphism (SShD) if multivariate measures of size are not used. Univariate studies may also overestimate the prevalence or direction of SSD when the sexes are strikingly different in shape, which may be an issue in taxa such as Orthoptera and other terrestrial arthropods where maximum body size is strongly constrained. Here we tested for the occurrence of both SSD and SShD in the badlands cricket Gryllus personatus (Orthoptera, Gryllinae). We measured four body size dimensions-maxillae span, head width, pronotum length, and mean hind femur length—and used multivariate methods to test whether male and female adult badlands crickets were sexually dimorphic in size and/or shape. All the univariate dimensions were sexually dimorphic, with males having wider heads and maxillae than females and females having longer pronota and hind femora than males, which indicates SShD. However, multivariate methods failed to detect SSD, instead confirming that the sexes primarily differ in body shape. We show how a simple ratio of head width to pronotum length captures SShD in badlands crickets and apply it to iNaturalist, a citizen science platform, to broaden our findings. We propose that orthopterists studying SD minimally measure head width, pronotum length, and hind femur length as a standard that will allow a more repeatable and generalizable assessment of the prevalence and direction of both SSD and SShD.

Keywords

body size, geometric mean size, Gryllinae, sexual shape dimorphism, sexual size dimorphism

Introduction

Intraspecific differences between males and females, or sexual dimorphism (SD), are common in sexually reproducing organisms (Darwin 1871, Andersson 1994, Fairbairn et al. 2007, Stillwell et al. 2010) and are thought to be caused by either intersexual competition for resources (Selander 1966), sex-specific equilibria (Blanckenhorn 2005), or some combination thereof. SD can take a variety of forms,

from differences in behavior (e.g., parental care, reviewed in Kokko and Jennions 2008), development (e.g., Esperk et al. 2007, reviewed in Hopkins and Kopp 2021), and immunity (e.g., Bagchi et al. 2021, reviewed in Kelly et al. 2018) to differences in overall body size (sexual size dimorphism, SSD; reviewed in Fairbairn et al. 2007) and body shape (sexual shape dimorphism [SShD], e.g., Table 1). These latter two are of special interest because they: 1) are relatively easy to measure, 2) can impact other dimorphisms (e.g., sex differences in mobility, reviewed in Herberstein et al. 2017) as well as important life history traits (e.g., fecundity, reviewed for spiders in Foellmer and Moya-Laraño 2007), and 3) unlike other dimorphisms, SSD and SShD are preserved in the fossil record making it possible to study their long-term evolution (e.g., Martins et al. 2020). In organisms such as arthropods that have an exoskeleton, maximum body size and therefore SSD may be evolutionarily constrained (reviewed in Whitman 2008 and references therein), placing greater selection pressure on the evolution of body shape and thus SShD.

The order Orthoptera (crickets, katydids, grasshoppers, and allies) display one of the highest degrees of SSD among insects (Stillwell et al. 2010), with most species displaying female-biased SSD (Hochkirch and Gröning 2008). Most of these instances of SSD are based on total body length as an index of body size, because it is a commonly reported metric of size in the taxonomic literature from which the bulk of these data were gleaned (Hochkirch and Gröning 2008). However, because total body length includes the relatively soft and flexible abdomen that can fluctuate in size, the rate of SSD should be interpreted with caution (Hochkirch and Gröning 2008). Furthermore, because these data rely on a single morphological dimension, conclusions about the extent and direction of SSD are confounded with SShD.

The badlands cricket, *Gryllus personatus* (Uhler 1864), is a midsized field cricket (Orthoptera, Gryllinae) inhabiting clay-type badlands in the southwest United States (Weissman and Gray 2019). As typical for most field crickets, males have specialized forewings (tegmina) that they rub together to produce song, and females have a long, thin ovipositor that they use to deposit fertilized eggs into soil (Weissman and Gray 2019). Beyond these sex-limited

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Table 1. Selected published descriptions of sexual shape dimorphism in the Orthoptera. Choice of what morphological dimension is considered the focal shape variable and what is considered a reference trait reflects the original publication and is presumably chosen to reflect a functional hypothesis. Obviously, this polarity can be reversed, in which case the direction of dimorphism would be reversed (e.g., female *G. pennsylvanicus* have longer hind femora than males for a given head width; see also figure S2 in Judge and Bonanno 2008).

Taxon	Shape Variable	Reference Trait	Direction	References
Caelifera				
Elasmoderus wagenknechti	forewing length, hindwing length	abdomen length	M>F	Cepeda-Pizarro et al. 2003
Gomphocerus sibiricus	foretibia girth	not specified	M>F	Valverde et al. 2018
Temnomastax spp.	tegmen length	hind femur length	F>M	da Silva Olivier and
	body length	tegmen length	F>M	Aranda 2018
Ensifera				
Acheta domesticus	head width	pronotum width	M>F	Walker et al. 2008
Gryllacropsis sp.	not specified	not specified		Tomar and Diwakar 2020
Gryllus pennsylvanicus	multiple mouthpart dimensions and	pronotum length, hind	M>F	Judge and Bonanno 2008
	head width	femur length		
Hemideina crassidens	head length, head width	hind femur length	M>F	Kelly 2006
H. maori	head width	hind tibia length	M>F	Gwynne and Jamieson
				1998
Pachyrhamma spp.	length of several antennal sensillae	not specified	various	Fea et al. 2019
P. waitomoensis	hind leg length	pronotum width	M>F	Fea and Holwell 2018
Zaprochiline katydid	auditory bulla size	not specified	F>M	Bailey and Simmons 1991

characteristics, there is no published account of any other sexually dimorphic morphology in the badlands cricket. Our main goal with this study is to test the hypotheses that male and female badlands crickets differ in body size and/or body shape (i.e., display SSD and/or SShD, respectively). We measured four morphological dimensions that are predicted to be sexually dimorphic and then used multivariate statistical methods to assess both SSD and SShD.

Methods

Study animals and care.-Individuals used in this study were the offspring of approximately 60 late instar nymphs (30 males, 30 females) supplied by David Gray (California State University, Northridge) from his laboratory colony of badlands crickets, originally collected from Winslow, Arizona, USA. Crickets were housed in conditions like those described in Judge and Bonanno (2008) at the Department of Biological Sciences, University of Lethbridge. Briefly, hatchlings were added to a large plastic bin (48 cm long, 35 cm wide, 31 cm high) containing layers of egg cartons for shelter, glass shell vials filled with water and stoppered with cotton for moisture, and ground cat food (Iams Original with Chicken; Iams, Mason, Ohio, USA) and ground rabbit food (Martin Little Friends; Martin Mills Inc., Elmira, Ontario, Canada) to provide a variety of food choice (cat food is high in protein, whereas rabbit food is high in fiber). Environmental conditions in the lab during rearing were 25°C, 70% relative humidity, and 12 hours light:12 hours dark daily light cycle. The badlands cricket was to be included in a larger comparative study of aggressive behavior, but this colony had to be euthanized (-20°C for 24 hours) when the study was ended because the second author accepted a fulltime faculty position at MacEwan University. All individuals that were in the colony were preserved separately in 70% ethanol for later measurement.

Measurement of morphology.—We measured five morphological dimensions in all individuals: head width, maxillae span, pronotum length, and both left and right femur length using a methodology similar to that of Judge and Bonanno (2008). Briefly, each individual was removed from their container and dissected to remove the head and both hind femora (at the juncture with the hind coxae) and placed in a standardized position submerged in 70% ethanol under a stereomicroscope (M5; Wild, Heerbrugg, Switzerland) with an attached phototube (Martin Microscope Company, Easley, South Carolina, USA) and digital camera (INFINITY 1-3C; Teledyne Lumenera, Ottawa, Ontario, Canada). We used INFIN-ITY CAPTURE v5.0.2 (Teledyne Lumenera) to capture three images: 1) the ventral head perpendicular to the transverse plane, 2) the dorsal thorax perpendicular to the frontal plane, and 3) the lateral femora perpendicular to the sagittal plane. These photographs were used to place landmarks using the programs tpsUtil and tpsDig2 (SB Morphometrics, https://sbmorphometrics.org/), and the landmarks were converted to linear dimensions using basic trigonometry in Excel (Microsoft Corporation, Redmond, Washington, USA). The landmarks used for each linear dimension are described in detail in Judge and Bonanno (2008, see Fig. 1 therein), and the image capture and morphological measurement procedures are explained in more detail in Dupuis et al. (2020). All photographs and the resulting measurement data used for our analysis are freely available at Dryad (https://doi.org/10.5061/ dryad.gjg2bvgkw).

Statistical analysis.—We reduced the number of morphological variables from five to four by using the mean of the left and right femur lengths. Then we used a multivariate general linear model with sex (male or female) as the independent variable and the four morphological variables (head width, maxillae span, pronotum length, and mean femur length) as dependent variables to test for sex-related differences in morphology. Kolmogorov-Smirnov tests were used to assess the normality of the residuals of all statistical analyses. We used IBM SPSS Statistics Version 28.0 (IBM Corporation, Armonk, New York, USA) for all analyses, which were carried out at a Type 1 error rate set at 5%.

Results

The head width, maxillae span, pronotum length, and left and right femur lengths of 167 (71 males and 96 females) adult *G. personatus* were measured. One male was excluded



Fig. 1. Average scores for adult male (filled circles) and adult female (open circles) badlands crickets (*Gryllus personatus*) on the first two components from a principal components analysis of the four measured variables: head width, maxillae span, pronotum length, and mean femur length. Error bars represent standard errors. See Table 3 for factor loadings.

from further analysis because he had deformed forewings, and 26 females were excluded using a random number generator to even the sample sizes for males and females at 70 apiece (Table 2). Kolmogorov-Smirnov tests for normality revealed that the residuals from the following statistical tests were normally distributed (all *p* > 0.083). A multivariate GLM with sex as the fixed factor and maxillae span, head width, pronotum length, and mean femur length as dependent variables revealed overall sexual dimorphism (*F*_{4,135} = 81.894, *p* < 0.001), although post-hoc tests showed that the direction of dimorphism depended on the trait measured. Adult males had wider maxillae spans (*F*_{1,138} = 54.506, *p* < 0.001) and wider heads (*F*_{1,138} = 10.492, *p* = 0.002), whereas adult females had longer pronota (*F*_{1,138} = 13.587, *p* < 0.001) and longer femora (*F*_{1,138} = 7.217, *p* = 0.008) (Table 2).

Given that the direction of sexual dimorphism varied across linear dimensions, it was not clear whether males and females

Table 2. Means \pm standard deviations (range in parentheses) of five measures of size in the badlands cricket (*Gryllus personatus*). Mean femur length is the average of left and right femora, and geometric mean size is the fourth root of the product of head width, maxillae span, pronotum length, and mean femur length. N = 70 for both males and females.

Measurement (mm)	Males	Females
Maxillae Span	4.87±0.47 (3.87-5.95)	4.38±0.30 (3.58-5.05)
Head Width	5.18±0.39 (4.21-6.06)	4.99±0.33 (4.11-5.85)
Pronotum Length	3.29±0.26 (2.49-3.78)	3.45±0.27 (2.66-3.98)
Mean Femur Length	9.90±0.68 (8.30-11.68)	10.22±0.73 (8.45-12.02)
Geometric Mean Size	5.35±0.40 (4.28-6.26)	5.27±0.36 (4.27-6.13)

Table 3. Results of the principal components analyses for adult badlands crickets (*Gryllus personatus*) including factor loadings, eigenvalues, and percent variance explained for PC1 and PC2.

Dimension	PC1	PC2
Maxillae Span	0.823	0.554
Head Width	0.955	0.247
Pronotum Length	0.843	-0.486
Mean Femur Length	0.912	-0.309
Eigenvalue	3.132	0.699
% Variance Explained	78.311	17.471

were dimorphic in just body shape or in both body shape and overall body size. We investigated sexual dimorphism in overall size by conducting two separate analyses. First, we calculated the geometric mean of the four morphological measures as an index of overall size (Mosimann 1970) and compared males and females using a t-test. Adult G. personatus were not sexually dimorphic in geometric mean size (adults: $t_{138} = 1.318$, p = 0.190). Second, we conducted a principal components analysis (PCA) to reduce the four measured variables to a limited number of uncorrelated principal components (PCs), where PC1 represents overall size and other PCs represent latent shape variables (Jolicoeur 1963, Cadima and Jolliffe 1996). Although only PC1 had an eigenvalue over 1, we also extracted PC2 (Table 3) because we had an a priori hypothesis regarding shape difference between the sexes. We tested for size and shape sexual dimorphism using separate t-tests. Adult *G. personatus* were dimorphic in PC2 ($t_{138} = 16.961$, p < 0.001) but not PC1 ($t_{138} = 0.888, p = 0.376$; Fig. 1).

To further explore adult sexual dimorphism, we conducted a discriminant function analysis (DFA) to find out whether there was a linear combination of our measured variables that could accurately predict the sex of individual adult *G. personatus*. The DFA resulted in a significant linear combination of measurements ($\chi^2_4 = 167.489$, p < 0.001) that successfully identified the sex of adult *G. personatus* 94.3% (66/70) of the time for females and 91.4% (64/70) of the time for males. The resulting canonical dis-

Table 4. Structure matrix from a discriminant function analysis distinguishing adult male and adult female badlands crickets (*Gryllus personatus*). Values represent the pooled within-groups correlations between discriminating variables and the standard-ized canonical discriminant function.

Variable	Function
Maxillae Span	0.403
Head Width	0.177
Pronotum Length	-0.201
Mean Femur Length	-0.147

criminant function was positively correlated with head width and maxillae span and negatively correlated with pronotum length and mean femur length (Table 4).

Finally, we wanted to test whether the SShD we detected in labreared badlands crickets was generalizable to the species. First, we calculated the ratio of head width to pronotum length. This ratio variable was sexually dimorphic in adults ($t_{138} = 11.520$, p < 0.001) and was highly positively correlated with both PC2 (r = 0.917, p < 0.001) and the discriminant function (r = 0.821, p < 0.001). To assess the generalizability of this ratio, we used the online natural history website iNaturalist to collect images of wild adult *G. per*- *sonatus* and measured the ratio of head width to pronotum length using the same procedure as above. We chose only observations that had 1) at least one photo taken from above and perpendicular to the frontal plane and 2) attained the status of "Research Grade", which meant that at least two people had agreed on the species-level identification with no dissenting opinions. This selection procedure resulted in 10 useable observations (9 females and 1 male). Because of the small sample size, we did not perform a statistical analysis comparing the head width:pronotum length ratios of wild crickets to lab-reared crickets. In wild crickets, as in lab-reared crickets, the male had a bigger ratio than females, and the ratios of wild crickets were less than the ratios of lab-reared crickets, although this difference was less than 5% (Fig. 2).

Discussion

In this study, we tested whether the badlands cricket, Gryllus personatus, displayed SSD, SShD, or both. Of the four morphological dimensions that we measured, all were highly positively correlated, and all were sexually dimorphic in adults but in contrasting directions. Adult females had longer hind femora and longer pronota but narrower heads and smaller maxillae spans than adult males (Table 2, Fig. 3), clearly indicating SShD. Because the direction of sexual dimorphism varied by morphological dimension, it was unclear whether badlands crickets were sexually dimorphic in overall body size. We addressed this question in two ways: 1) we compared adult males and females on the geometric mean of all measured variables (GMS), and 2) we used principal components analysis (PCA) to extract a multivariate measure of size (principal component 1, PC1) and an uncorrelated PC2, which can be interpreted as a measure of shape (Jolicoeur 1963). Adult males and females differed on PC2 but not PC1 or GMS, indicating that adult badlands crickets are sexually dimorphic for shape but not size. To further explore SD in the badlands cricket, we conducted a DFA that extracted a linear combination of the measured variables that accurately identified the sex of adult crickets at least 91% of the time. The resulting discriminant function had factor loadings that closely mirrored those of PC2, namely that head width and maxillae span loaded in the opposite direction as pronotum length and mean femur length. These patterns are consistent with the divergent univariate patterns of sexual dimorphism (Table 2) and describe a pattern of SShD whereby adult males have larger heads and mouthparts and adult females have larger thoraxes and hind legs.

Males of many animals have relatively bigger heads and mouthparts than females, including lizards and snakes (e.g., Becker and Paulissen 2012, King et al. 1999, Kratochvíl and Frynta 2002), amphibians (e.g., Katsikaros and Shine 1997, Zhang et al. 2020), fish (e.g., Laporte et al. 2018), mammalian carnivores (e.g., Gittleman and Van Valkenburgh 1997), beetles (e.g., Marlowe et al. 2015). and orthopterans (e.g., Kelly 2006, Walker et al. 2008, Judge and Bonanno 2008) (see Shine 1989 for a review of sexually dimorphic trophic structures). Amongst orthopterans, larger head size in males is thought to be the result of sexual selection because males compete for access to female mates by grappling with their mouthparts (Kelly 2006, Judge and Bonanno 2008). In the fall field cricket, G. pennsylvanicus, males with proportionately larger heads were more likely to win aggressive contests, but only if those contests escalated to grappling with mouthparts (Judge and Bonanno 2008). Like most Gryllus species (Jang et al. 2008, Bertram et al. 2011), adult male badlands crickets compete aggressively with each other for females using their heads and mouthparts



Fig. 2. Average values for the ratio of head width to pronotum length for lab reared (open squares, N = 70 for both sexes) and wild (filled squares, N = 1 male and 9 females) badlands crickets (*Gryllus personatus*). Error bars represent 95% confidence limits for lab reared crickets and \pm one standard deviation for wild females.



Fig. 3. Matrix scatterplot of the four measured variables: maxillae span (MS), head width (HW), pronotum length (PL) and mean femur length (MFL), and geometric mean size (GMS) for male (filled circles, N = 70) and female (open circles, N = 70) badlands crickets (*Gryllus personatus*). All variables have been log transformed (LG) to facilitate comparison of scaling relationships. Note that the axis labels are all contained along the diagonal so that each is both an x-axis label for any plots above and below and a y-axis label for any plots to the left and right. Thus, every pairwise combination of variables is plotted twice, with each variable appearing on the x-axis on one side of the diagonal and on the y-axis on the other.

Table 5. Studies from a special issue on body size in Orthoptera that measured multiple homologous morphological dimensions in both males and females, where, in principle, it would have been possible to evaluate both SSD and SShD. Columns indicate the taxa studied, the number of morphological dimensions measured, whether SSD was evaluated (and if so whether a univariate or multivariate measure of body size was used), conclusions regarding the direction of SSD, and whether SShD was evaluated.

Taxa	Measurements	SSD?	SSD Pattern	SShD?	Reference
Caelifera					
Chorthippus vagans	4	Yes (U)	F>M	-	Ciplak et al. 2008
Cornops aquaticum	3	Yes (U)	F>M	Yes	Adis et al. 2008
Dactylotum variegatum	2	Yes (U)	F>M	Yes	DeBano et al. 2008
Dichroplus pratensis	6	No	-	Yes	Bidau and Martí 2008b
	6	Yes (U)	F>M	-	Bidau and Martí 2008a
D. vittatus	6	Yes (U)	F>M	-	Bidau and Martí 2008a
Gomphocerinae (8 spp.)	10	Yes (U)	F>M	-	Picaud and Petit 2008
Oedipoda miniata	4	Yes (U)	F>M	-	Ciplak et al. 2008
Oedipodinae (4 spp.)	10	Yes (U)	F>M	-	Picaud and Petit 2008
Podisma sapporensis	8	No	-	-	Sugano et al. 2008
Romalea microptera	2	Yes (U)	F>M	-	Huizenga et al. 2008
	10	Yes (U)	F>M	Yes	Vincent and Lailvaux 2008
Ensifera					
Pholidoptera frivaldskyi	5	Yes (U)	F>M	-	Fabriciusová et al. 2008
Poecilimon birandi	4	Yes (U)	F>M	-	Ciplak et al. 2008
P. thessalicus	3	Yes (U)	F>M	-	Lehmann and Lehmann 2008
Roeseliana roeselii	5	No	-	-	Berggren 2008
Stenopelmatus sp.	4	Yes (U)	No	Yes	Weissman et al. 2008

(D. Gray, pers. comm.), suggesting that the sexual dimorphism in head size relative to pronotum length may also be influenced by sexual selection through male-male competition. Conversely, the observed SShD may also reflect differences between the sexes in mobility, with females evolving longer legs and larger thoraxes (i.e., increase stride length and leg muscle mass, respectively) because sexually active female field crickets search for sedentary singing males (Alexander 1961) all the while attempting to avoid predation, which may be strongly influenced by jumping ability (e.g., Ercit et al. 2014). There are several mechanisms which may result in sex-specific optima for morphological (or other) traits (Shine 1989, Blanckenhorn 2005), and future research should investigate sex-specific mobility patterns, diet, burrow digging, and aggression and the adaptiveness of variation in head and mouthpart size versus thorax and leg size.

Even though every morphological trait we measured in adult badlands crickets was sexually dimorphic in univariate analyses, our multivariate analysis failed to detect any evidence of SSD. This contrasts with the almost universal female-biased SSD in over 1500 species of Orthoptera reported in a recent review (Hochkirch and Gröning 2008). This difference is not surprising given that we used a multivariate approach to measuring body size, whereas, by necessity, Hochkirch and Gröning were constrained to use total body length, a univariate measure of body size that is widely reported in the taxonomic literature that formed the basis of their dataset (Hochkirch and Gröning 2008). We did not measure total body length because of the inherent variability in this measurement due to the effects on abdomen size of nutritional status, oocyte growth, and preservation artefacts (Hochkirch and Gröning 2008), and so our results are not directly comparable. However, our study raises questions as to the true extent of SSD and how often SSD is conflated with SShD. In a recent special issue on body size in Orthoptera (Whitman and Vincent 2008), of 13 studies that measured multiple homologous morphological variables in both males and females (representing 27 species), only 5 evaluated SShD, and all 5 found evidence supporting sex differences in body shape (Adis et al. 2008, Bidau and Martí 2008b, DeBano 2008, Vincent and Lailvaux 2008, Weissman et al. 2008). Furthermore, of the 9 studies that evaluated SSD, all but one concluded that females were larger than males; none used a multivariate measure of size but instead evaluated SSD separately for one or more individual measurements (Table 5). It is unclear whether a multivariate measure of these studies, but until more studies assess both SSD and SShD, we will not know how often these two sexual dimorphisms confound or complement each other.

Although we failed to detect SSD using multivariate methods, the conclusion that adult badlands crickets are not sexually dimorphic for body size should be viewed with some caution as it may depend on the choice of measured dimensions. Choosing an appropriate index of body size is no simple task (Fairbairn 2007), and this is precisely because of shape variation within species, including 1) SShD, 2) polymorphisms (e.g., alternative male morphs in isopods, Shuster 1987), 3) age-related changes in body shape, and 4) condition-dependent effects on body shape (e.g., parasite effects). Many researchers use a single morphological dimension as an index of size (Fairbairn 2007). This practice has the advantage of convenience, standardization, and comparability, whereas multivariate measures of size may not be convenient and can be sample-dependent and therefore not comparable between studies (e.g., principal components). In insects, head width is often used as an index of size (Fairbairn 2007). Because of mounting evidence of relative head size difference between males and females in Orthoptera (e.g., Kelly 2006, Walker et al. 2008, Judge and Bonanno 2008, this study, KAJ unpubl. data) it is becoming increasingly obvious that, for studies of orthopterans where sex is a factor of interest, using head width as an index of body size is no longer appropriate. Failing any consensus on a single measure of size, we recommend a geometric mean of head width, pronotum length, and hind femur length. This measure is 1) multivariate, 2) representative (i.e., the three dimensions represent two body regions

and three morphological axes), 3) convenient (i.e., only three dimensions per individual that can be measured on both live and preserved specimens), 4) universal (i.e., all Orthoptera have these three structures, which is not the case for wings), 5) comparable (i.e., not sample dependent like PC-based size measures), and 6) insensitive to variation in individual condition and damage/preservation artifacts (i.e., does not include weight or dimensions of the abdomen, and component dimensions are rigid and shrink little with drying). Even if individual studies chose to base their analyses on a different index of size, widespread measurement of head width, pronotum length, and hind femur length would greatly facilitate comparative analyses of both SSD and SShD.

SShD variation can be measured in a variety of ways, including intersexual comparisons of 1) regression slopes, 2) second and higher order principal components, 3) values on a discriminant function separating males and females, and 4) ratios of different dimensions to an index of body size (e.g., shape ratios; Mosimann 1970). Ideally SShD studies should incorporate more than one of these methods, and to the extent that different methods result in similar conclusions, shape ratios offer a useful comparative function that is absent from the other multivariate, sample-dependent methods. Ratios are scale-independent and so can be calculated from any image regardless of magnification. In the badlands cricket, results from PCA, DFA, and a simple comparison of the ratio of head width to pronotum length all suggested the same conclusion: that males have relatively wider heads than females. We then used the simple ratio method to measure SShD in a sample of images of badlands crickets posted by citizen scientists to iNaturalist. The head width/pronotum length ratios of these wild crickets were within 5% of the averages of our lab-reared individuals, and the degree of SShD was similar in wild and lab-reared badlands crickets. It is worth noting that machine learning methods for image recognition, such as the one used by iNaturalist to suggest species identification, rely on scale-independent characteristics such as ratios of different dimensions.

In conclusion, we found evidence of SShD in adult badlands crickets: females had relatively longer hind legs and pronota than males, who had relatively wider heads and maxillae spans than females. A variety of multivariate methods failed to detect SSD, and although we cannot (nor wish to) claim that male and female adult badlands crickets are the same size, we do suggest that our results are cause to revise how body size is typically measured in arthropods in general and Orthoptera in particular. The geometric mean of three body dimensions—head capsule width, pronotum length, and hind femur length—has both the properties of universality, sample independence, comparability, and is multivariate. The widespread adoption of this body size measurement by orthopterists would open up enormous possibilities for comparative assessments of the prevalence and direction of both SSD and SShD.

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A new species of *Neoleva* (Caelifera, Acridoidea, Acrididae, Gomphocerinae) from Central Tanzania

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Abstract

A new species of *Neoleva* Jago, *N. magna* sp. nov. is described from Central Tanzania. A key to all species of *Neoleva* is presented.

Keywords

East Africa, grasshopper, taxonomy

Introduction

Neoleva Jago, 1996 was erected by Jago (1996) with *N. mega* Jago, 1996 from Ethiopia as the type species, and another three species from Kenya were included. *Neoleva* are stocky brachypterous Gomphocerinae. The antennae are longer than the head and the pronotum together. Males have a rounded subgenital plate and simple cerci. There is a marked sexual dimorphism, the females being considerably larger than the males (Fig. 1). Males show a contrasting color pattern with an orange dorsal abdomen and orange parts on the hind femora, while females are duller in color. In males a pair of white spots at the anterior margin of the pronotum is present and a pair of angulate cream to white bands on the meso- and metanotum. The epiphallus has a strong bridge and a narrow interspace (Jago 1996). *Neoleva* are a geophilous species found in habitats ranging from semi-desert to savanna woodlands.

In this paper, a new species, *N. magna*, is described from the Mpwapwa District in Central Tanzania.

Material and methods

Material studied.—All available specimens of the four species of *Neoleva*, including the type specimens present in the Natural History Museum London, UK, were studied and compared to specimens of the new species, *Neoleva magna* sp. nov. from Tanzania.

Genital preparations.—For genital preparations, specimens were relaxed in water, the phallus extracted manually, macerated in 5% KOH, then neutralized in 5% acetic acid.

Photographic procedure.—The macerated epiphallus was photographed with an Olympus Tough TG 6 Camera, and the images were processed using the stacking program supplied with that camera.

Measurements.—The body length refers to the body length of the insect from head to the tip of the abdomen. Additionally, the total length according to Jago (1996), taken from the frons to the tips of the folded posterior femora, is given.

Depositories.—BMNH: Natural History Museum London, UK. CCH: Collection of Claudia Hemp.

Results

Neoleva magna sp. nov.

https://zoobank.org/F6206387-0AA6-4460-929F-40C31722D26C

Material examined.—Holotype: TANZANIA • \Im ; Mwpapwa District, Changalawe Hill; 6°53'47"S, 36°02'46"E; miombo woodlands, ca 750 m; March 2015, C. Hemp leg.; BMNH. Paratypes: TANZANIA • 1 \Im ; same data as holotype; BMNH •2 \Im , 2 \Im , same data as holotype; CCH • 3 \Im , 1 \Im ; Msaze village near Gulwe; 6°31'23"S, 36°22'12"E; 1000 m a.s.l.; March 2017 and March 2020; C. Hemp leg; CCH.

Description.—Male. General coloration: A speckled pattern of gray, dark, and white patches with abdomen, inner sides of the hind femora, and apical parts of the tibiae orange. Tegmina light brown in costal area, darker brown above with some dark spots around the media area (Fig. 2). Pronotum with well-developed

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Fig. 1. Types of *Neoleva* species. A. Male holotype of *N. mega*; B. Types of *N. bufoides*, left male, right female; C. Types of *N. robertsoni*, left male, right female; D. Types of *N. kevani*, left male, right female.

pair of white spots at anterior margin and angulate white lines on meso- and metazona (Fig. 3D). Ventral parts of lateral pronotal lobes white or creamy (Fig. 3A). Lunules of hind femora dark with a postgenicular white band both on femur and tibia (Fig. 2A). Remaining part of outer side of hind femora greyish or creamy with three dark fasciae on dorsal ridge. These fasciae extend into inner parts of the hind femora. Fore and mid legs speckled white or greyish and black. *Head and antennae.* Antenna about 1.5 times longer than head and pronotum, about 13 mm. Frontal ridge flat coarsely punctate on upper part and with a medial ocellus at about the middle of the ridge (Fig. 3C).

Pronotum and legs. Pronotum crossed by three deep sulci, the posterior one separating the mesozona from the metazona continuing deep into the lateral pronotal lobes with an abrupt angle about in the middle of the pronotal lobes and then running anteriorly almost to the anterior margin (Fig. 3A). A medial carina is faint on pro- and mesozona but well-developed on the metazona. The tegmina reach to about the middle of the abdomen and are tectate with elevated veins along the whole length.

Abdomen. Supra-anal plate triangular, at tip appendiculate (Jago 1996; compare his figs 109–112, p. 80 and Fig. 3 B). Cerci are short and slightly laterally compressed (Fig. 3 B).

Internal genitalic morphology. Ancorae of epiphallus very weakly sclerotized and comparatively small. Bridge narrow. Inner and outer lophi typical for *Neoleva* (Fig. 4).

Female. Larger and stouter than the male (Fig. 2B), color pattern duller but generally similar to male having a pair of white dots at the anterior pronotal margin and angular white lines at the posterior part of the pronotum (Fig. 5A). Some females are uniformly brown while others also have orange hind femora. Ovipositor valves short; dorsal surfaces of upper valves concave, forming an almost cup-like structure with dark sclerotized margins. Ventral valves with slightly undulating margin, this margin also dark sclerotized with more acute tips (Fig. 5B).

Measurements. (mm)

	Males (N=4)	females (N=6)
Head width	4.2-4.4	5.7-6.1
Interocular Distance	1.0-1.1	1.1-1.2
Posterior femur length	13.0-12.8	17.5-18.8
Posterior femur width	3.7-3.8	4.7-5.2
Tegminal length	7.1-7.6	10.1-10.9
Pronotum length	3.3-3.5	4.8-5.2
Pronotum width	4.7-4.9	6.7-7.8
Body length	17.7-18.5	26.2-26.5
Total length*	18.5-22.0	24.5-29.5

*Frons to tips of folded posterior femora.

Differential diagnosis.—Neoleva magna sp. nov. is the largest known species in the genus, males having a total length of about 18.5 to 22 mm, following Jago (1996) who measured the total length (frons to tips of folded posterior femora). All other known species of Neoleva are smaller. The females are also larger than those of the known other species, although means overlap (see Table 1). Male N. magna sp. nov. resemble N. bufoides Jago, 1996 and N. robertsoni Jago, 1996 in having a white or creamy ventral margin of the pronotal lobes. In those species, however, the outer sides of the hind femora are more or less uniformly orange to red but are light grey or whitish in N. magna sp. nov. in which only the ventral and inner sides of the hind femora are vivid orange. N. mega Jago, 1996 and N. kevani Jago, 1996 appear to have much duller coloration. However, living specimens of these taxa have not been seen, and for N. mega, only the male holotype is known. N. magna sp. nov. also has an appendiculate supra-anal plate as described by Jago (1996) for N. kevani and N. mega. In contrast to the other known species of Neoleva, N. magna sp. nov. has tectate folded tegmina, while in the other species, the wings are more evenly shaped and more closely appressed to the abdomen. Internally, the male



Fig. 2. Neoleva magna sp. nov. A. Male; B. Female.



Fig. 3. *Neoleva magna* sp. nov., morphological details of male. A. Lateral view on head and pronotum; B. Dorsal view on abdominal apex with supra-anal plate; C. Face; D. Head and pronotum, dorsal view.



Fig. 4. Epiphallus of Neoleva magna sp. nov.

epiphallus of *N. magna* differs from the other three species in its relative size, the distance of the ancorae to each other and the orientation and size of the lophi.

Song.—Unknown.

Etymology.—From latin: *-magnus* = big, because it is the largest species of the genus so far.

Habitat.—Ground dweller on open patches within miombo woodlands and along forest margins with sparse vegetation.

Distribution.-Tanzania, Mpwapwa District.



Fig. 5. Female Neoleva magna sp. nov. A. Dorsal view of head, pronotum, and spread wings B. Lateral view on abdominal apex.

Species /characters	Tegmina	Total length male	Total length females	Color ventral lateral pronotal lobes	Supra-anal plate
N. magna sp. nov.	tectate	>18.5 mm	24.5-29.5 mm	white/creamy	apical appendix
N. robertsoni	lobes	<18.5 mm	22.6-25.7 mm	white/creamy	tapered
N. bufoides	lobes	<18.5 mm	22.2-24 mm	white/creamy	strongly tapered
N. kevani	lobes	<18.5 mm	Not known	brown/black	apical appendix
N. mega	lobes	<18.5 mm	Not known	brown/black	apical appendix

4

Table 1. Morphological characters distinguishing Neoleva species.

Key to Neoleva males (adapted from Jago 1996)

1 Tegmina tectiform; male body length (head to tips of folded hind femora) >18.5 mm; Central Tanzania, Mwpapwa DistrictN. magna sp. nov. 1′ 2 2´ Lateral pronotal lobes light brown or black ventrad 4 3 Inner lophi widely spaced. Supra-anal plate weakly tapered, lacking a transverse sulculus, with extensive melanic area and pair of darker melanic spots in basal two-fifth. (Kenya, Meru Region).....N. robertsoni Jago, 1996 31 Inner lophi comparatively closely spaced. Supra-anal plate strongly tapered, with a clear transverse sulculus and with two weak darkly pigmented areas distal to sulculus (Kenya, Lysamais

area).....N. bufoides Jago, 1996

Inner pair of lophi comparatively small, dorso-lateral angles of lophi produced and lateral margins constricted to form a waist. Abdomen vivid orange red at maturity. Supra-anal plate with strong marginal darker pigmentation and broad apical appendix. (S. Kenya)......*N. kevani* Jago, 1996 Inner pair of lophi comparatively large, dorso-lateral angles not greatly produced and central section of lateral margins not constricted. Abdomen dark brown to red and black. Supra-anal plate with small apical appendix and rather uniform darker brown pigmentation throughout lightening at margin (Ethiopia)......*N. mega* Jago, 1996

Discussion

Neoleva species are geophilous according to habitat information provided in Jago (1996) and on the labels. No information is available for the holotype Ethiopian male of *N. mega. N. bufoides* (*Acacia*



Fig. 6. Distribution of Neoleva species. Source of map: Google maps.

shrub; sparse vegetation, *Acacia* thicket, banks of dry riverbed, see Jago 1996), *N. robertsoni* (thicket and herbs), and *N. kevani* (desert grass and thorn bush) are all species that were caught in savanna woodlands on patches with sparse vegetation or in semi-desert areas. *N. magna* sp. nov. occupies a similar habitat in miombo woodlands where it was found at two localities: South of Dodoma at the edge of Changalawe hill covered with sparse miombo forest and at a similar hill near Gulwe not far from the district city Mpwapwa. Hemp and Heller (2019) gave more information on the localities near Gulwe and Changalawe Hill, also listing syntopic Orthoptera for these areas. As already pointed out in Hemp and Heller (2019) Miombo woodlands are vanishing rapidly, mostly due to recurrent burning and transformation into agricultural fields. *N. magna* sp. nov. is probably also under pressure since it seems to be bound to thickets and forest edges of miombo woodlands.

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A new *Floritettix* (Orthoptera, Acrididae, Melanoplinae) from the Bombing Range Ridge, Florida, U.S.A.

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Abstract

Floritettix are endemic to the North American Coastal Plain. Here I describe a new species, *Floritettix phlox* **sp. nov**., from the Bombing Range Ridge in central Florida. This species appears to be restricted to the heavily burned scrub habitat on this small ridge. This species is described based on morphological and biogeographical evidence.

Keywords

Biodiversity, fire, grasshopper, islands, Lake Wales Ridge, sand ridges

Introduction

In 2015, while conducting field surveys at Avon Park Air Force Range with John Barone, Reed Noss, and Steve Orzell, I collected a *Floritettix* Otte, 2014 that upon later microscopic examination did not fit the described species in a recently published revision of Otte (2014). More specimens were acquired during subsequent field expeditions to Florida and specifically Avon Park Air Force Range. Comparisons of these specimens with other *Floritettix* indicate that this is a new species that may be endemic to the scrub community of the Bombing Range Ridge. If so, then this would represent the first animal known to be endemic to the ridge.

Floritettix is one of the five acridid genera endemic to the southeastern United States (along with *Aptenopedes* Scudder, 1878, *Eotettix* Scudder, 1897, *Floridacris* Otte, 2014, and *Gymnoscirtetes* Scudder, 1897) and is Floridian in distribution, meaning that it is endemic to Florida and the southern portions of the adjacent states of Alabama and Georgia (Hill 2018). Otte (2014) established *Floritettix* with 13 species (six new; seven formerly *Aptenopedes*). Specimens from the Bombing Range Ridge were not examined in that study as access to the ridge has been limited since 1941, after the establishment of Avon Park Air Force Range. Most of the ridge itself occurs on the training range, where Air Force personnel gain experience firing aircraft mounted explosive ordinances, which results in frequent fires (Orzell and Bridges 2006). However, not all

Floritettix are inhabitants of scrub environments. For example, a cursory examination of Otte's (2014) maps indicate that *Floritettix hubbelli* (Hebard, 1936) have broad distributions that span much of peninsular Florida. My own sampling indicates that it typically inhabits moister flatwoods adjacent to the scrub environments and hyperseasonal prairies across south Florida.

Floritettix is differentiated by characters associated with the male genitalia. These morphological characters have been long used for species delineations in the Melanoplinae and have been further supported by molecular evidence (Hubbell 1932, Otte 2014, Hill 2015, Woller 2017, Huang et al. 2020). Here I provide the first key to the genus and a description of the new species.

Materials and methods

All but one of the specimens used in this study were collected by staff of the Mississippi Entomological Museum (MEM) and are deposited there. A single specimen was found in the insect collection at the Archbold Biological Station (ABS). Comparisons are made with *Floritettix nigropicta* and *F. hubbelli*, two species that occur in close geographical proximity to the new species. Habitus and internal genitalia photographs were taken with a Leica Z16 stereoscope equipped with a Leica DFC420 camera and imaged at different stages during dissection. Images were automontaged with the Leica Application Suite. Measurements, made with a reticle mounted inside a Leica MZ12.5 stereomicroscope, were taken as follows:

Body length—Dorsally from the fastigium vertices to the distal end of the genicular lobe of caudal femur in a parallel plane with the abdomen.

Pronotum length—Dorsally, along the median carina.

Cercus length—Laterally, maximum possible measurement of the left cercus.

Cercus basal width—Laterally, along the point of attachment from the dorsal to ventral margin.

Mid cercus width—Laterally, at the mid-length of the left cercus. Cercus apex width—Laterally, along the distal end.

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J. G. HILL

Results

Key to male *Floritettix*

- 2' Dorsal valves narrow, with moderate sheathing, not appearing bilobed and extending beyond the length of the ventral valves (Fig. 1B); rami shorter and broad; known only from Manatee County on the west coast of Florida.......F. ocilla Otte, 2014

- 4 Body grayish green with lateral and dorsal thoracic sulci strikingly black colored (sometimes lost in pinned specimens) (Fig. 6J); apices of ventral valves broad basally, apically acute (Figs 1C, 6C–G); found on the Lake Wales Ridge in Central Florida.......*F. nigropicta* (Hebard, 1936)
- 5 Apices of ventral valves semifalciform (Fig. 1E); lateral striping on the pronotum orange and black; subgenital plate and pallium gray to grayish pink; found in Marion, Orange, or Putnam Counties in northeast Florida*F. holotamico* Otte, 2014
- 5′ Apices of ventral valves broadly rounded (Fig. 1D); lateral striping on the pronotum white subgenital plate and pallium pink found in Highlands or Polk County in central Florida*F. phlox* sp.nov.
- 6 In lateral view, sheath of the aedeagus ovoid (Fig. 1F–J)......7
- 7' In lateral view, sheath of the aedeagus broadly ovoid (Fig. 1H–J)9
- 8 In dorsal view, the sheath of the aedeagus is constricted near their midpoint (Fig. 1F); apices of ventral valves acute (Fig. 1F); from Brevard or Indian River Counties in east central Florida F. floridana Otte, 2014
- 9 In dorsal view, the sheath of the aedeagus is wider than long (Fig. 1H); ventral valves narrow; from Orange and Polk Counties in central Florida......*F. hadjoi* Otte, 2014
 9' In dorsal view, the sheath of the aedeagus is longer than wide (Figs 11–J)......10
 10 In ventral view, ventral valves more evenly triangular (Fig. 11); from the west coast of Florida from Hillsborough County south to Lee

12 In lateral view, the sheath is very shallowly oblong; ventral valves abruptly taper to a point (Fig. 2D); from southeast Florida..... 12'In lateral view, the sheath is oblong; ventral valves shorter and obtusely triangular (Fig. 2A); from Osceola and Seminole Counties in east central FloridaF. osceola Otte, 2014 13 Sheath more evenly rounded apically, not constricted medially (Fig. 2B); found on the Atlantic Coast of Florida in Duval, Flagler, and Volusia Counties, east of the St. Johns River F. simplex (Hebard, 1936) Sheath more acute apically, constricted medially (Fig. 2D); found in 13' northeast Florida in Flagler, Nassau, Putnam, St. Johns, and Volusia

Taxonomy

Family Acrididae MacLeay, 1821 Subfamily Melanoplinae Scudder, 1897

Genus Floritettix Otte, 2014

Type species.—*Floritettix aptera.*

Floritettix phlox sp. nov.

https://zoobank.org/27893934-AA54-4081-9C26-75AE9C07BD1C Figs 1D, 3, 4A–C, 5A–J

Material examined.—**Holotype**: USA • 3; FLA, Highlands Co., Avon Park Air Force Range; 27.5788 °N, -81.2516 °W; 16 June 2015; J. G. Hill, J. Barone, R. Noss, S. Orzell leg; low oak scrub, Bombing Range Ridge; MEM. **Other specimens examined**: USA • 13; Florida: Polk Co., Avon Park Bombing Range; 24 September 1991; M.E. Deyrup leg ; ABS • 23, 19; Avon Park AFB; 27.6481 °N, -81.2747 °W; 20 June 2019; J.G. Hill, B.S. Dunaway leg; low oak scrub, Alpha Range; MEM • 13; same data except; 27.6481 °N, -81.2736 °W; J.G. Hill leg.; low oak scrub, Alpha Range; MEM • 43; Avon Park AFB; 27.6744 °N, -81.2861 °W; 5 October 2021; M.J. Thorn leg.; scrubby flatwoods/low oak scrub; MEM • 13; same data except; 27.6729 °N, -81.2874 °W; 5 October 2021; M.J. Thorn leg.; scrubby flatwoods and low oak scrub; MEM • 29; same data except; J.G. Hill leg.; MEM • 43, 19; 27.6836 °N, -81.2897 °W; 5 October 2021; J.G. Hill leg.; MEM.

Diagnosis.—Differing from other Floritettix in the shape of the male genitalia, which has the sheath produced over the dorsal valves as two large adjacent, rounded-subquadrate lobes with a decided concavity caudad, ventral valves that slightly curve dorsally with broadly rounded apices, and a distinct geographic distribution (Figs 1D, 5C–J). Can be separated from *F. nigropicta* based on the green coloration of the body (gravish in F. nigropicta), the lack of black-colored body sutures, and the shape of the male genitalia that are rounded ventral valve apices and more rounded to subquadrate dorsal valve/ sheath complex (Figs 1D, 6C-J), and Floritettix holotamico by the color of the dorsal lateral pronotal stripe (white in *F. phlox*, orange in F. holotamico) and the shape of apices of the ventral valves (round in F. phlox and falcate in F. holotamico). Floritettix hubbelli occurs in the hyperseasonal Florida dry prairies that surround the Bombing Range Ridge. Floritettix phlox can be distinguished from F. hubbelli by the coloration of the post ocular and dorsal stripes (yellow in F. *hubbelli* and white with a pink border in *F. phlox*) and the very different shapes of the internal male genitalia (Figs 1A, D, 6C-G, 7C-G).



Fig. 1. Partial illustrations of male genitalia of *Floritettix* species showing the cingulum, rami, sheath of aedeagus, and distal portion of dorsal and ventral valves in lateral, dorsal, and ventral (left to right) views; C-cingulum, RC-ramni of cingulum, S-sheath of aedeagus, DV-dorsal valves, VV-ventral valves (gray). A. F. hubbelli; B. F. ocilla; C. F. nigropicta; D. F. phlox sp.nov.; E. F. holotamico; F. F. floridana; G. F. aptera; H. F. hadjoi; I. F. calusa; J. F. borealis.



Fig. 2. Partial illustrations of male genitalia of *Floritettix* species showing the cingulum, rami, sheath of aedeagus, and distal portion of dorsal and ventral valves in lateral, dorsal, and ventral (left to right) views; C-cingulum, RC-ramni of cingulum, S-sheath of aedeagus, VV-ventral valves (gray). A. *F. coquinae*; B. *F. osceola*; C. *F. simplex*; D. *F. saturiba*.

Male measurements.—(in mm; n = 14) Body length 16.6–19.6 (mean = 18.1); pronotum length 3.3–3.7 (mean = 3.5); hind femur length 8.6–9.8 (mean = 9.1); cerci length 0.6–1.0 (mean = 0.8); basal width of cercus 0.3–0.5 (mean = 0.5); mid-cercal width 0.2 3–0.3 (mean = 0.2); cerci apex width 0.1 (mean = 0.3).

Female measurements.—(in mm; n = 3) Body length 21.4–24.0 (mean = 22.5); pronotum length 4.4–5.1 (mean = 4.7); hind femur 10.5–12.2 (mean =11.5).

Description.-External morphology.-Body of medium size (Figs 4A-C, 5J). Head moderately large in proportion to the body (especially in females), having the face strongly oblique, fastigium broad in dorsal view and produced anterior to the eyes; eyes large and prominent, elongate-oval. Antennae filiform and slender, with joints somewhat flattened. Pronotum with dense shallow punctures throughout, distinctly widening from apex to base, female widening only on the metazona. Median carina low, cut only by the principal sulcus; lateral carinae absent; prozona over twice as long as the metazona; with a feebly rounded front margin, metazona with hind margin sub-truncate or broadly emarginate; lateral lobes sub-vertical, nearly twice as long as deep, narrowing ventrally, tegmina vestigial, reduced to minute inconspicuous scales. Tympanum large; prosternal spine cylindrical and slender with a blunt apex, hind femora slender; slightly surpassing the abdomen in the male and reaching the base of the ovipositor in the females. Abdomen compressed and carinate; male supra-anal plate short, triangular with lateral margins weakly convergent, with its margins rounded and reflexed and with a short basal median groove. Cercus simple, triangular about twice as long as broad, tapering to an acute apex (Fig. 3A, B). Furcula are a pair of minute divergent lobes, slightly longer than wide. Sub-genital plate short, not tuberculate, with a narrow chitinous ring that is slightly thicker in the medially, tapering laterally. Pallium a large fleshy rounded flap that rests over the base of the male genitalia.

Phallic structures.—Sheath produced over the dorsal valves as two large proximo-lateral processes as in other species in the genus, but more specifically as two large adjacent, rounded-subquadrate lobes with a decided concavity caudad, the ventral pair



Fig. 3. Male Floritettix phlox sp.nov. Illustrated by Ashley Rude Baker.



Fig. 4. Photos of live *Floritettix phlox* sp.nov. and the plant communities where specimens were collected. **A.** Lateral view of a live male; **B.** Dorsal view of a live male; **C.** Lateral view of live female; **D.** Low oak scrub at the type locality; **E.** Scrubby flatwoods; **F.** Scrubby Flatwoods.

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Fig. 5. *Floritettix phlox* A. Dorsal view of terminalia; B. Lateral view of terminalia; C. Dorsal view of phallic complex; D. Lateral view of aedeagus; G. Caudal view of the aedeagus; H. Dorsal view of epiphallus; I. Caudal view of epiphallus; J. Habitus.



Fig. 6. *Floritettix nigropicta* A. Dorsal view of terminalia; B. lateral view of terminalia; C. Dorsal view of phallic complex; D. Lateral view of phallic complex; E. Dorsal view of aedeagus; F. Lateral view of aedeagus; G. Caudal view of the aedeagus; H. Dorsal view of epiphallus; I. Caudal view of epiphallus; J. Habitus.

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Fig. 7. *Floritettix hubbelli* A. Dorsal view of terminalia; B. Lateral view of terminalia; C. Dorsal view of phallic complex; D. Lateral view of aedeagus; F. Lateral view of aedeagus; G. Caudal view of the aedeagus; H. Dorsal view of epiphallus; I. Caudal view of epiphallus; J. Habitus.

of parameres situated ventro-proximad; adjacent, directed distad and slightly curving dorsal to their broadly rounded apices. The epiphallus is of the typical melanoploid shape, with lophi, ancorae, and an undivided bridge. More precisely, epiphallus with a concave bridge, bilobed lophi, convexly curved lateral plates subrectangular in shape with an angular anterior lobe and a long, acute caudal tip; ancora closely set, triangular, taper to a point, and decurved ventrally.

Coloration.—Overall greenish in life can fade to yellow in specimens (Figs 3, 4A–C, 5J). Antenna light, white in life, turning darker in specimens. Antennal crescent complete. Head, thorax, and abdomen green. Males with a well-defined, white or pinkish-white post-ocular stripe that extends to the caudal edge of the metazona and a moderately broad white to pinkish-white stripe margined with a suffusion of black extending medio-longitudinally on the dorsum of the abdomen. Females typically lacking the post-ocular stripes and with only a faint indication of the abdominal stripe.

Mouthparts, supra-anal plate of male and the genicular area of the hind femora pinkish-brown, genicular arches of latter black. Female with mouthparts pinkish. Fore and middle tibia faintly glaucous blue, tarsi gray. Caudal tibia black basally then glaucus blue, caudal tarsi rich pink in males, females similar, but tibia can be purplish. (Figs 3, 4A–C, 5J)

Distribution.—Floritettix phlox is known only from the Bombing Range Ridge in Highlands and Polk Counties in Central Florida (Fig. 8).

Etymology.—"phlox" from the Greek word for flame, in reference to the frequent fires that occur on the Bombing Range Ridge that maintains the habitat for the species. Further, the terminalia and mandibular areas are pink like *Phlox* flowers.

Habitat.—Floritettix phlox appears to be restricted to the low oak scrub and scrubby flatwoods on the Bombing Range Ridge (Fig.



Fig. 8. Map of Florida showing the location of the Bombing Range Ridge with an inset showing the Bombing Range Ridge, the Lake Wales Ridge, and the collecting localities of *Floritettix phlox* sp.nov. Map data 2022 (C) Google.

3D–F). Searches in the adjacent Florida dry prairies yielded only specimens of *F. hubbelli*, and *F. nigropicta* seems to replace it on the adjacent Lake Wales Ridge. *Floritettix phlox* is typically found on scrubby oaks with which it blends in quite well with (Fig. 3B).

Discussion

The sand ridges of Florida have been long recognized areas of endemism (Devrup 1990). These well-drained ridges and uplands are ancient islands that served as refuges during periods of higher sea level in the Miocene, Pliocene, and Pleistocene (McNeill 1957). During that time, the islands were areas of evolutionary divergence for populations separated by the sea but, due to their edaphic conditions, still function as islands after the water retreated from the surrounding lowlands. The resulting habitat islands support a xerophytic plant community called Florida scrub that is characterized by low, evergreen sclerophyllous oaks (Quercus ssp.), various shrubs, palmettos, sand pine (Pinus clausa), and numerous forb species with interspersed areas of bare sand. Florida scrub is a fire-maintained community, and in the absence of fire, the oaks grow dense and tall, often reducing grasshopper diversity and abundance (J. G. Hill unpublished data). The scrub on the Bombing Range Ridge is maintained by frequent fires resulting from explosives used in military training exercises as well as prescribed burns.

Studies on the speciation and biogeography of grasshoppers inhabiting the scrub communities of these sand ridges has focused on species belonging to the large genus Melanoplus Stål, 1873 (Hubbell 1932, Deyrup 1990, Woller 2017) and has largely ignored the five genera endemic to the North American Coastal Plain (Hill 2018). Otte (2014) shows only county-level distributions and is of limited use in determining fine-scale distributional patterns. However, based on museum records and my own field studies, it appears that F. nigropicta is endemic to the Lake Wales Ridge and F. phlox to the Bombing Range Ridge. The Bombing Range Ridge rises from the Osceola Plain, reaching elevations of 38.1-44.2 m.a.s.l., and is thought to be a relict marine sand bar. In places, it is separated by as little as 2 km from the much larger Lake Wales Ridge (Fig. 6), but the two do not share identical floras and faunas (Orzell 1993, Branch and Hokit 2000). The Arbuckle Creek lowlands flow between the two ridges likely provides a dispersal barrier for flightless scrub-inhabiting insects.

The discovery of *Floritettix phlox* demonstrates that there is still a need for biodiversity exploration in the scrub communities on the Florida peninsula. Furthermore, given that *Floritettix* is Florididan in distribution, phylogenetic and biogeographic studies would likely yield results as equally interesting or greater than that of *Melanoplus*. Indeed, I have begun gathering specimens of all the species in the genus in ethanol for population-level study.

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Alien mantids in a tropical paradise: First record of the giant mantis Sphodromantis viridis (Insecta, Mantodea) for the Canary Islands (Spain)

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Abstract

The presence of adults, nymphs, and oothecae of the African giant mantis *Sphodromantis viridis* Forsskål is for the first time recorded on the island of Tenerife, the largest and most populated of the Canary archipelago, an autonomous Spanish community in the Atlantic Ocean. The discovery took place in a popular water park rich in non-native plants in the municipality of Costa Adeje. It is possible that anthropogenic movements, particularly the massive movement of tourists and goods that continually cross this small archipelago, aided the dispersal to the area of this mantis, which for some years has also been expanding in many areas of the Mediterranean basin. A hypothesis on the ecological impact and the possible invasiveness of this extremely adaptable species is discussed and supported by the large number of sightings of oothecae and specimens that are, fortunately, still confined within the west coast of the island.

Keywords

Alien species, expansion, giant mantis, human-mediated introduction, Tenerife

Introduction

Sphodromantis Stål, 1871 is a genus of large sized mantids characterized by a robust body, well-developed flight organs in both sexes, thick head with round eyes and a small tubercle at the base of the antennae, and pronotum longer than fore coxae with well-marked supracoxal dilatation (Battiston et al. 2010). The 37 species currently included in this genus (Anderson 2022) live in various biotopes characterized by a warm climate, from equatorial forests to semi-desert regions. Their distribution includes the entire African continent, the Middle East, and part of southern Europe (Roy 2010).

Sphodromantis viridis Forsskål, 1775 (often known by the common name of giant African mantis) is the species of the genus with the northernmost distribution. It is common from the Middle East to the sub-Saharan region of some Central African countries

(Battiston 2020), while in Europe, it was believed that a stable population existed only in the south of Spain, where this mantis was reported a century and a half ago (Bolívar 1876). In recent years, there has been a significant expansion of the distribution range of this insect not only in other locations on the Iberian Peninsula (Marabuto et al. 2014, Cabanillas 2017, Oliveira and Ferreira 2019) but also on the Balearic Island of Mallorca (Canyelles and Alomar 2006), in Sardinia (Ruzzante and Leo 2012), Sicily and Greece (Battiston et al. 2020), and Croatia (Martinović et al. 2022). The data provided by these studies have shown that this species of mantis, despite being xerothermophilous and more closely linked to its distribution range than others of its own family, can be considered a generalist predatory insect capable of spreading rapidly even in heavily anthropized places, increasing the possibility of a human-mediated introduction in areas adjacent to its original habitat, including islands. Until now, the phenomenon seemed to involve mainly the Mediterranean area, but in this article, the presence of Sphodromantis viridis is reported for the first time on the island of Tenerife, the largest island of the Canary archipelago, located in the Atlantic Ocean off the African coast. This is also the first record of this mantis on a non-Mediterranean island.

Materials and methods

The production of this work started with the finding of *Sphodromantis viridis* in the south of Tenerife by one of the authors, who came to the Canary Islands for an expedition. All sightings occurred within the boundaries of the "Aqualand Costa Adeje" water park. One adult specimen and eight oothecae were collected (Fig. 1), carefully examined using the guidelines contained in Brannoch et al. (2017), and photographed with a Samsung Galaxy S10e equipped with a clip-on 100 mm HD macro lens for smartphone camera and a Nikon Coolpix b500. The collected material was identified and analyzed using the scarce but detailed scientific literature regarding this mantis. Further research was conducted using taxonomic, citizen science, and online platforms (Inaturalist,

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Fig. 1. Presence of *Sphodromantis viridis* within the Aqualand Costa Adeje Water Park. A. Adult female B. Ootheca laid on *Nerium oleander* L. C. Old, damaged ootheca D. Fertile ootheca with a recently hatched nymph. Photos by Alessio De Martino.

GBIF), and from tourists and workers within the water park. Observations of the plants in the park were made by the authors both directly on site and by looking at photos of the area available on some websites (Google maps, Aqualand.es). Identifications were conducted with the help of the Plants of the World website of the Kew Royal Botanic Gardens.

Results

Material examined.—SPAIN • 1 \bigcirc , 1 sub-adult \Diamond , 5 unsexed nymphs and 13 oothecae; Canary Islands, Tenerife Island, Santa

Cruz de Tenerife province, Adeje municipality; Costa Adeje seaside community; 28°04'75"N, 16°43'68"W; 286 m a.s.l.; 24 Aug. 2022; Alessio De Martino leg.; found among the plants of "Aqualand Costa Adeje" water park, collected by hand and deposited in Antonio Fasano's collection.

The only adult specimen collected, an 8.2-cm female, was quickly identified based on the following characters: large head with rounded eyes and frontal sclerite barely longer than high; pronotum with metazone/prozone ratio > 2; supracoxal dilation well marked; strong fore legs, three coxal spines with a yellowish plate at the base, black femoral spines only at the tip; flight or-


Fig. 2. Main features used for the identification of *S. viridis*. A. Pronotum with well-marked supracoxal dilatation. B. Head with rounded eyes and a tubercle near the base of antennae. C. Robust fore legs with coxal spines with yellowish plates. D. Bright white stigma on the tegminae. Photos by Alessio De Martino.

gans well developed, tegminae with clearly visible whitish stigma; and hind wings hyaline (Fig. 2). (Battiston et al. 2010, Battiston et al. 2019). The eight oothecae collected turned out to be from different periods: Some were approximately 1–2 years old, damaged, and hatched, and others were close to hatching. In addition to the collected material, many other oothecae and some young nymphs were observed, including a sub-adult male. Almost all the specimens and the oothecae were high on the plants used to decorate the park, which made it difficult to collect much material but confirmed the life and oviposition preferences of this species on

stems, branches, and leaves (Younes and Gabre 2003). The plants present in the park area are mostly non-native, some of which are common in areas where this mantis is widespread and all which are well adapted to warm and tropical climates (Table 1). The presence of numerous other insects was also noted, confirming the creation in this highly anthropized area of a microenvironment suitable for a large proliferation of *Sphodromantis viridis*. However, it has not been possible to trace a possible date on which this mantis arrived on the island, but it is hypothesized that it was a sudden appearance followed by a rapid expansion favored by the **Table 1.** Non-native plants observed in the park with their native range and the presence/absence of oothecae and specimens of *Sphodromantis viridis*.

Plant	Origin	Presence of S. viridis	
Agave attenuata	Mexico	No	
Agave tequilana	Mexico	No	
Aloe arborescens	Southern Africa	No	
Aloidendron dichotomum	Namibia, South Africa	No	
Codiaeum variegatum	Indonesia, Malaysia, Oceania	Oothecae	
Crassula ovata	Southern Africa	No	
Eleagnus × submacrophylla	Hybrid of Asian species	Oothecae	
Eucalyptus sp.	Oceania, Philippines	No	
Euonymus sp.	Asia, Europe	No	
Excoecaria cochinchinensis	China, Southeast Asia, Taiwan	Oothecae, specimens	
Hibiscus rosa-sinensis	Vanuatu	Oothecae	
Iresine diffusa f. herbstii	Peru	Oothecae	
Lantana camara	Central and tropical America	No	
Musa sp.	Tropical Asia, Oceania	Oothecae	
Metrosideros excelsa	New Zealand	No	
Nerium oleander	Africa, Asia, Europe	Oothecae, specimens	
Polygala sp.	Cosmopolitan	No	
Pandanus utilis	Mascarene islands	Oothecae	
Schinus molle	South America	No	
Tillandsia xerographica	Central America	No	
<i>Washingtonia</i> sp.	Mexico, United States	Specimens	

environmental conditions of Tenerife, characterized by a subtropical climate (Juan et al. 2000).

Discussion

In recent years, there has been a rapid change in the spread of praying mantids around the world, with many native species expanding north due to higher global temperatures and many alien species introduced by humans in numerous territories, following the main trade routes such as the one that starts from Asia to get to Europe (Shcherbakov and Govorov 2020). Most alien mantids are members of the Mantidae family: extremely adaptable generalist predators of considerable size and capable of proliferating even in environments profoundly modified by human activities. Sphodromantis viridis also has these characteristics, with the difference that this mantiss more linked to xerothermophilic contexts and seems to expand in a more restricted way than other genera of mantis with which it is related, favoring places with a climate and environment similar to those of the species' origin. The beginning of the expansion of this mantis in the territories that are currently part of its distribution range probably occurred in the Pleistocene (La Greca 1966) during the end of the glaciation and warming of North Africa and the Euro-Mediterranean area. The subsequent isolation of some populations in different areas of Africa has allowed these mantids to fragment into different subspecies, each characterized by specific morphological and morphometric characteristics (La Greca and Lombardo 1987). The number and validity of the subspecies of S. viridis have always been the subject



Fig. 3. Map of *Sphodromantis viridis* observations in the Aqualand Costa Adeje water park; red triangles: damaged or hatched oothecae, green triangles: unhatched oothecae, yellow stars: unsexed nymphs, red star: sub-adult male, purple square: adult female. Base image from Google Maps 2022 [©] Google.

of discussion among scholars, and with the new distributions, it is probable that many are no longer considered valid precisely because the isolation of their populations is lacking (Battiston, personal communications). The discovery of these African giant mantids for the first time on the Canary Islands confirms the ability of these insects to spread and create stable populations even on islands located in the tropical belt of the Atlantic Ocean. The observation of many oothecae and specimens of all stages within the limit of the water park, if they are not the result of multiple introductions, could suggest rapid growth not controlled by any type of efficient predator, with a potentially incisive impact on the colonized area (Battiston 2020), which could end up hosting a huge number of specimens and oothecae in a relatively small space, at the same way as the invasion of Hierodula tenuidentata Saussure, 1869 in the countryside of Northern Italy (Battiston et al. 2018). The Canary Islands are the habitat for ten native species of praying mantids (Wieland 2014); many of these are endemic, while others, such as Blepharopsis mendica Fabricius, 1775 or Hypsicorypha gracilis (Burmeister, 1838), are also widespread in northern Africa and the Middle East (Battiston 2016a, Battiston 2016b). The island of Tenerife appears to be a central volcanic island, as suggested by some of its features such as large calderas, but the previous existence of land bridges that may have allowed some animals of the African continent on this archipelago is not excluded (Rothe 1974). Therefore, it cannot be totally excluded that Sphodromantis viridis arrived on the island of Tenerife on its own, even if this scenario is extremely unlikely. However, analyzing the possible causes of the spread of this species in Sardinia, Battiston et al. (2017) highlighted how the passive transport of oothecae is improbable due to the time it would take to reach the coasts of an island, a period not compatible with the annual life cycle of this mantis. Even the possibility that a female arrived alone in flight is not plausible since they are not mantids suitable for flying over such long distances. The most likely cause of the introduction is anthropogenic. This is supported by the fact that the first findings of this species took place in a tourist place characterized by a continuous flow of means of transport, goods, and people, with the presence of many non-native plants also coming from the Euro-Mediterranean area. Thanks to the data collected, it is possible to confirm the presence of Sphodromantis viridis in a restricted area of Tenerife (Fig. 3), but

it is not yet possible to establish whether its spread is truly limited. The authors hope that future studies will be conducted to continue monitoring the dynamics of this giant mantis population in the middle of the ocean to better understand their spread and whether they are a threat to native ecosystems.

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A replacement name for *Bostra* Stål (Insecta, Phasmida, Diapheromeridae), a junior homonym of *Bostra* Walker (Insecta, Lepidoptera, Pyralidae)

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Abstract

Bostra Stål (Phasmida, Diapheromeridae) is a junior homonym of *Bostra* Walker (Lepidoptera, Pyralidae). The replacement name *Bostranova* Villet **nomen nov.** is proposed for *Bostra* Stål, and new combinations are proposed for the species-group names currently included in that phasmid genus.

Keywords

homonymy, name change, new combination, nomenclature, replacement name, stick insects

Introduction

Francis Walker (1863) described a monotypic genus of pyralid moths using the name *Bostra*. This genus now contains about 50 species from Africa, Turkey, and India. Twelve years later, Carl Stål (1875) used the same name in describing a genus of diapheromerid stick insects that now contains 27 species distributed from Brazil to Mexico. Walker died in 1874 (Carrington 1874), the year before Stål's publication, and Stål died a few years later in 1878 (Reuter 1878). Stål's publications focused on Hemiptera, especially from southern Africa, and also included Orthoptera, Phasmida, Coleoptera, and Hymenoptera (Reuter 1878) but not Lepidoptera. For these and perhaps other reasons, this homonymy was discovered by neither author.

The phasmid genus was last augmented in 2012 with a single species from Tobago (Langlois and Bellanger 2012). Prior to that, Werner (1929) added one new species, Hebard (1919) added one, Redtenbacher (1908) added 24 species, Rehn (1904) added three, Griffini (1896) added one, and Brunner von Wattenwyl, Scudder, and Westwood each described a species that was subsequently transferred to *Bostra* (Stål 1875, Kirby 1904, Rehn 1904, Redtenbacher 1908). Several species placed in *Bostra* (e.g. *Bostra crudelis* (Westwood, 1859), *Bostra dorsuaria* Stål, 1875, *Bostra podagrica* Stål, 1875, *Bostra martini* Griffini, 1896, *Bostra scabrinota* Redtenbacher, 1908, *Bostra tridenticulata* Redtenbacher, 1908, *Bostra imperialis* Redtenbacher, 1908, *Bostra ibaguena* (Giglio-Tos, 1910)

and *Bostra trinitatis* Werner, 1929) were later associated with various other genera as new taxon concepts developed (e.g., Zompro 2001, Conle et al. 2011, Figueiredo de Araujo and Senna Garraffoni 2012, Hennemann and Conle 2021). The genus was diagnosed and redescribed in 2001 (Zompro 2001). The homonym posed by *Bostra* was noticed by the lepidopterist Roger Kendrick (personal communication to P. D. Brock), and Paul Brock recorded this discover in a scrutiny note in 2009 in the Phasmida Species File Online (Brock et al. 2023, http://phasmida.speciesfile.org/Common/basic/Taxa.aspx?TaxonNameID=1202238), but it has remained unaddressed. No junior synonym of *Bostra* Stål, 1875 was traced (cf. Brock et al. 2023) that could serve as a replacement name under Article 60b of the International Commission on Zoological Nomenclature (ICZN) (ICZN et al. 1999).

In line with the requirements of Article 60 of the International Code of Zoological Nomenclature (ICZN et al. 1999), a replacement name for Stål's taxon is proposed here to relieve this situation.

Methods

In forming a replacement name, consideration was given to the cultural aspects of nomenclature raised by Gillman and Wright (2020) and to the needs of the users of scientific names (Garnett and Christidis 2017, Thomson et al. 2018). Attention was therefore given to interpreting Stål's intention in naming *Bostra*.

To ensure that the proposed replacement name was not itself a junior homonym, four global on-line nomenclatural databases were consulted: the Phasmida Species File Online (http://Phasmida.SpeciesFile.org), the Catalogue of Life (https://www.catalogueoflife.org/), the Encyclopedia of Life (https://eol.org), and the Global Biodiversity Information Facility (https://www.gbif. org). To some extent, these are cross-referenced to one another but also to other nomenclatural database initiatives, giving them an extensive collective reach (Garnett et al. 2020).

To evaluate the consequences of this nomenclatural act, a contemporary species list was compiled from examination of the literature (e.g., Kirby 1904, Rehn 1904, Redtenbacher 1908, Zompro 2001,

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Abbreviation	Repository
ANSP	Academy of Natural Sciences, Philadelphia, Pennsylvania,
	USA
ETHZ	Erdgenössische Technische Hochschule-Zentrum, Zurich,
	Switzerland
FLYB	Frédéric Langlois and Yannick Bellanger collection, France
HNHM	Hungarian Natural History Museum, Budapest, Hungary
MHNG	Muséum d'Histoire Naturelle, Geneva, Switzerland
MNCN	Museo Nacional de Ciencias Naturales, Madrid, Spain
MNHN	Muséum National d'Histoire Naturelle, Paris, France
NHMUK	The Natural History Museum, London, United Kingdom
NHMW	Naturhistorisches Museum am Wien, Wien, Austria
SMNS	Staatliches Museum für Naturkunde, Stuttgart, Germany
USNM	National Museum of Natural History, Washington D.C.,
	USA
ZMUH	Universität von Hamburg, Zoologisches Institut und
	Zoologisches Museum, Hamburg, Germany

 Table 1. Explanations for abbreviations of names of repositories of type material.

Conle et al. 2011, Figueiredo de Araujo and Senna Garraffoni 2012, Langlois and Bellanger 2012). To facilitate future taxonomic review, additional taxonomic and nomenclatural information was gleaned from published museum catalogues (Brock 1998, Zompro and Brock 2003, Zompro 2005, Brock et al. 2016, Delfosse et al. 2019).

Results

Taxonomy

Family Diapheromeridae Kirby, 1904

Genus Bostranova Villet, nomen nov.

Bostra Stål, 1875: 6, 13. Type species, by original monotypy: Bacteria turgida Westwood, 1859.

Bostranova Villet, nomen nov. for Bostra Stål, 1875, not Walker, 1863.

nec Bostra Walker, 1863: 123 (Walker 1863), = Therapne Ragonot, 1890: xciii (Ragonot 1890), synonymized by Leraut 2003: 123.

Type species.—Bacteria turgida Westwood, 1859, inherited from replaced name.

Discussion

It was decided to base the replacement name on Stål's original name to minimize users' frustration with the change (Garnett and Christidis 2017, Thomson et al. 2018), and to explain why the issue of cultural imperialism in nomenclature (Gillman and Wright 2020) has not strongly affected this particular nomenclatural action. The name is derived from the original genus *Bostra* and a suffix formed from the Latin word '*novum*' ('new' in English), having the sense of "the new *Bostra*", which is aligned with Stål's original apparent intention to commemorate a particular Middle Eastern town. Bostra (or Bosra or Busra; Ancient Greek: Bóστ@a; Latin: Colonia Bostra, Nova Trajana Bosra; Turkish: Eski Şam; Arabic (x=v,v)) was a commercial and administrative city of the Nabataean kingdom at the northern end of the Wādī al-Sirḥān trade route in southern Syria (32°30'53"N, 36°28'48"E) and was the capital of the Roman province of Arabia under Trajan from 106 A.D. and headquarters of the Legio III Cyreniaca (Dentzer-Feydy et al. 2014). It is unclear why Walker or Stål used this name for their genera, but no alternative etymology was found by internet searches. However, Stål named over 1000 genera and regularly used Classical names for them, e.g., the names of the cities Macynia, Banasa, and Stagira, and the names of the Romans Carausius, Statilia, and Arulenus, are all commemorated in Stål's insect genera. It would have been apposite to use a name from the organism's geographical range, as William L. Distant did with his new taxa only 30 years after Stål's publications, but it seems unlikely that, in 1875, Stål had access to the type of information that Gillman and Wright (2020) point out as useful to consider when forming scientific names for organisms.

Perhaps whimsically, it is also hoped that these insects walk with a gait and rhythm that resembles the 'bossa nova' Latin dance style that originated within their geographical distribution.

The replacement name's grammatical gender is feminine. This nomenclatural action results in the new combinations (comb. nov.) listed in Table 2.

Table 2. List of new combinations (comb. nov.) resulting from the replacement name *Bostranova* Villet **nomen nov.**, with the original genus of each species, its type repository and the nature of its type material, and its country of occurrence as recorded by Stål (1875), Rehn (1904), Redtenbacher (1908) and Figueiredo de Araujo and Senna Garraffoni (2012) were also compiled; the abbreviations for repositories are explained in Table 1.

Bostranova arcuata (Redtenbacher, 1908: 408) comb. nov. (Bostra Stål, nec Walker) [NHMW, holotype ♀ nymph] Espirito Santo, Brazil (Brock 1998)

- Bostranova bifida (Redtenbacher, 1908: 412) comb. nov. (Bostra Stål, nec Walker) [NHMW, holotype ♀ ?nymph] Venezuela (Brock 1998)
- Bostranova championi (Redtenbacher, 1908: 410) comb. nov. (Bostra Stål, nec Walker) [NHMUK, holotype ී] Cahabon, Vera Paz, Guatemala (Brock et al. 2016)
- Bostranova deplanata (Redtenbacher, 1908: 411) comb. nov. (Bostra Stål, nec Walker) [MHNG, holotype ♀] Cuba (Zompro and Brock 2003)
- Bostranova exigua (Scudder, 1875: 278) comb. nov. (Bacteria Berthold) [ANSP, holotype ♂] Peru
- Bostranova incompta (Rehn, 1904: 57) comb. nov. (Bostra Stål, nec Walker) [USNM, holotype 3] San Carlos, Costa Rica
- Bostranova innocens (Brunner von Wattenwyl, 1907: 324) comb. nov. (Dyme Stål) [NHMW, holotype ♂] Marcapata, Peru
- Bostranova jaliscensis (Rehn, 1904: 514) comb. nov. (Bostra Stål, nec Walker) [ANSP, holotype ♂] Jalisco, Tuxpan, Mexico
- *Bostranova lobata* (Redtenbacher, 1908: 408) **comb. nov.** (*Bostra* Stål, *nec* Walker) [NHMUK, holotype \Im] Chiriqui, Panama (Brock et al. 2016)
- Bostranova magistralis (Redtenbacher, 1908: 410) comb. nov. (Bostra Stål, nec Walker) [NHMUK, holotype ♀] Panzos, Vera Paz, Guatemala (Brock et al. 2016)
- Bostranova magnifica (Redtenbacher, 1908: 410) comb. nov. (Bostra Stål, nec Walker) [SMNS, at least one ♀, missing] Brazil

Bostranova margaritata (Redtenbacher, 1908: 412) comb. nov. (Bostra Stål, nec Walker) [MNHN, holotype ♀] Sierra del Nayarit, Mexico (Delfosse et al. 2019)

- Bostranova mirata (Redtenbacher, 1908: 410) comb. nov. (Bostra Stål, nec Walker) [MNCN, at least one ♂, missing] Guatemala
- Bostranova nuptialis (Redtenbacher, 1908: 410) comb. nov. (Bostra Stål, nec Walker) [MHNG, at least one ♂, missing] Guyana (Zompro and Brock 2003)
- Bostranova obtusecornuta (Redtenbacher, 1908:409) comb. nov. (Bostra Stål, nec Walker) [NHMW, MHNG, ZMHB, syntypes 3 ♂♂, 2 ♀♀] Guatemala and Costa Rica (Brock 1998, Zompro and Brock 2003, Zompro 2005)

- Bostranova procoppi (Redtenbacher, 1908: 411) comb. nov. (Bostra Stål, nec Walker) [HNHM, at least one ♀, destroyed] Mexico
- Bostranova pruinosa (Redtenbacher, 1908: 411, pl. 19: 3) comb. nov. (Bostra Stål, nec Walker) [NHMW, holotype ♀] Bahia, Belmonte, Brazil (Brock 1998)
- Bostranova reductedentata (Redtenbacher, 1908: 409) comb. nov. (Bostra Stål, nec Walker) [ETHZ, syntypes 2 ろう] Pará, Brazil
- Bostranova remiformis (Rehn, 1904: 58) comb. nov. (Bostra Stål, nec Walker) [USNM, holotype ♀] Piedras Negras, Costa Rica
- Bostranova saussurei Redtenbacher, 1908: 411) comb. nov. (Bostra Stål, nec Walker) [HNHM, at least one ♀, destroyed] Mexico
- Bostranova similis (Redtenbacher, 1908: 412) comb. nov. (Bostra Stål, nec Walker) [NHMUK, holotype ♀] Jalisco, Mexico (Brock et al. 2016)
- Bostranova submutica (Redtenbacher, 1908: 409) comb. nov. (Bostra Stål, nec Walker) [NHMW, holotype ♂] Venezuela (Brock 1998)
- Bostranova tabida (Redtenbacher, 1908: 410) comb. nov. (Bostra Stål, nec Walker) [ZMUH, holotype ♀] Espirito Santo, Brazil
- Bostranova tobagoensis (Langlois & Bellanger, 2012: 98) comb. nov. (Bostra Stål, nec Walker) [MNHN, FLYB, holotype ♀, paratypes 3 ♂♂, 2 ♀♀] track 3.2 km west of Castara – Plymouth road, Tobago (Langlois and Bellanger 2012, Delfosse et al. 2019)
- Bostranova tridenticulata (Redtenbacher, 1908: 411) comb. nov. (Bostra Stål, nec Walker) [NHMUK, holotype ♀] Guerrero, Acaguizolta, Mexico (Brock et al. 2016)
- Bostranova turgida (Westwood, 1859: 28, pl. 8: 4, 9) comb. nov. (Bacteria Berthold) [NHMUK, lectotype ♂, paralectotype ♀] Venezuela (Brock et al. 2016)
- Bostranova vacca (Redtenbacher, 1908: 408) comb. nov. (Bostra Stål, nec Walker) [NHMW, holotype \Im] origin unknown (Brock 1998)

Conclusion

There has been a fair amount of taxonomic traffic in and out of this genus, and new genera (e.g., *Oncotophasma* Rehn, 1904, *Alienobostra* Zompro, 2001, and *Caribbiopheromera* Zompro, 2001) have absorbed species once assigned to *Bostra* Stål (Zompro 2001). It is hoped that replacing this homonym will attract attention to the genus and that the information summarized in Table 2 will facilitate its comprehensive revision.

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Annelize Lloyd and Suncana Bradley attracted attention to the nomenclature of *Bostra* through their enquiries about South African tent caterpillars; Rhodes University provided computing and digital resources for resolving the matter; and Paul Brock and Judith Marshall (The Natural History Museum, London) very kindly improved the manuscript. All are thanked for their generous contributions.

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Geographic variation in the calling songs and genetics of Bartram's roundwinged katydid *Amblycorypha bartrami* (Tettigoniidae, Phaneropterinae) reveal new species

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Abstract

Previous work on Bartram's round-winged katydid, Amblycorypha bartrami Walker, found inconsistencies in song variation across the species' range. Individuals of purported populations of A. bartrami from sandhills across the southeastern US were collected, recorded, and their genes were sequenced to better understand their population structure and evolution. Significant differences in songs, morphology, and genetics were found among populations from Alabama (AL), Georgia (GA), North Carolina (NC), and South Carolina (SC), and they differed from those of individuals collected from the type locality in Florida (FL). Males from all populations produced songs composed of a series of similar syllables, but they differed in the rates at which syllables were produced as a function of temperature. At temperatures of 25 °C, the calling songs of males from populations in northern AL and GA were found to have the highest syllable rates, those from SC had the lowest rates, and those from NC were found to produce songs with doublet syllables at rates that were intermediate between those of males from FL and those of AL and GA. These song differences formed the basis for cluster analyses and principal component analyses, which showed significant clustering and differences in song spectra and morphology among the song morphs. A Bayesian multi-locus, multispecies coalescent analysis found significant divergences from a panmictic population for the song morphs. Populations from GA and AL are closely related to those of A. bartrami in FL, whereas populations from NC and SC are closely related to each other and differ from the other three. Large river systems may have been important in isolating these populations of flightless katydids. Based on the results of our analyses of songs, morphology, and genetics, three new species of round-winged katydids from the southeastern coastal plain and piedmont are described.

Keywords

massively parallel sequencing, multi-locus multi-species coalescent model, new species

Introduction

The round-headed katydids of North America (Amblycorypha Stål, 1873) consist of three species groups-oblongifolia, rotundifolia, and uhleri-that differ in morphology and size (Rehn and Hebard 1914, Walker 2004). Walker et al. (2003) reviewed the rotundifolia complex and described two species, Amblycorypha bartrami Walker, 2003 and A. alexanderi Walker, 2003, based on differences in their calling songs and ecology. All three species in the complex from the eastern United States are cryptic, with calling song being the only useful character for distinguishing between A. rotundifolia, A. alexanderi, and A. bartrami. Bartram's roundwinged katydid, A. bartrami, occurs primarily in xeric longleaf pine and turkey oak habitats in the southeastern United States. During Walker et al.'s (2003) research, it became apparent that populations of supposed A. bartrami near Aiken, South Carolina differed significantly in calling songs from typical A. bartrami from Florida. The specimens were designated A. nr bartrami at the time. Other populations (e.g., in North Carolina) also exhibited song anomalies that indicated more thorough investigations were needed. Two of us (TGF and JDS) undertook a broader examination of A. bartrami across its range, including collecting DNA and using molecular data to understand the population structure and evolution within this species. Because the song rates of A. nr bartrami in South Carolina are similar to those of A. parvipennis, whose populations are all west of the Mississippi River, we also include data from populations of A. parvipennis in Arkansas and Missouri.

In this paper, we describe the variation in calling song, morphology, and genetics of populations of purported *A. bartrami*. We present the first molecular phylogenetic data from widespread populations in the *rotundifolia* complex, which show significant

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divergence among them. Members of the *rotundifolia* group, including *A. bartrami*, are flightless, which probably influences gene flow among populations. Therefore, we discuss the phylogeography of *A. bartrami* and how our genetic results relate to isolation and spatial population structure, particularly concerning river drainages and fragmentation of the longleaf pine habitat. Clustering analyses across populations also detected previously unidentified population differences in song and morphology. The significant genetic, acoustical, and morphological variation we discovered reveal new species that were, at one time, considered *Amblycorypha bartrami*.

Materials and methods

Fieldwork.—Fieldwork occurred mostly at night, and katydids were collected by listening for and finding males as they called or by searching vegetation for males and females using headlights. In some cases, males and females were collected during the day using sweep nets in areas and from vegetation likely to harbor katydids. Katydids were housed in $10 \times 10 \times 10$ cm cages (either clear plastic or screened) with ad libitum water and food (apple, lettuce, oats, or a dry high-protein artificial diet; Gwynne 1988). For some individuals, we removed a hind leg that was stored at -80°C for DNA extraction and sequencing (see below). Collection sites were typical A. bartrami habitats of longleaf pine, turkey oak sandhills distributed throughout the southeastern US, including Alabama (AL) $(3\stackrel{\circ}{\ominus}: 1\stackrel{\circ}{\downarrow}$, Cleburne Co.), Florida (FL) $(4\stackrel{\circ}{\ominus}: 1\stackrel{\circ}{\downarrow}$, Liberty Co.), Georgia (GA) (43: 42, Gordon Co.), North Carolina (NC) (93: 1 \bigcirc , Richmond Co.), and South Carolina (SC) (4 \bigcirc : 3 \bigcirc , Aiken Co. [SCA]; 63: 82, Edgefield Co. [SCE]; 63: 42, Georgetown Co. [SCG]). Collection sites for A. parvipennis include Arkansas (AR) (53° ; 3° , Faulkner Co.) and Missouri (MO) (33° : 0° , Shannon Co.). Because the songs of GA and AL specimens were found to have similar features and females from each population duetted with males from each population, their data were combined in many analyses and were designated GAL.

Acoustic recordings and analyses.—Calling songs of free-ranging males in the field or caged males in the laboratory were recorded with Sennheiser ME66 shotgun microphones and either a Tascam DAP-1 DAT recorder or a Marantz PMD-670 solid-state recorder. The sampling rate for the digital recordings was either 44 or 48 kHz. Time and frequency characteristics of the calling songs were determined with Audacity 2.3 or using the seewave package in R (Sueur et al. 2008, Sueur 2018, R Core Team 2020, RStudio team 2020). To reduce noise and echoes, laboratory recordings were made with microphones 0.5 m from the caged males with the substrate between the male and microphone covered with Sonex acoustic foam.

The songs of *A. bartrami* are relatively uniform, and a complete cycle of wing movement (syllable = phonatome, Baker, and Chesmore 2020) is indicated by repeated patterns in the time waveforms of the songs (Walker et al. 2003). Syllable rates of katydids vary with temperature, and these relationships differ among species, making syllable rate a distinguishing character (Walker 1975). For consistency, one of us (TGF) measured syllable rates during the sustained portion of calling songs (see also Walker et al. 2003). When possible, the rates were based on 10 syllables. However, in some recordings, the sustained series had fewer than 10 syllables at consistent rates. In those cases, the rates were determined based on 4–8 (typically 5) syllables. We also included recordings from previous work in our analyses (Shaw et al. 1990, Walker et al. 2003).

Spectral variation among populations was also examined at two different temporal levels: 30 s of calling song and for individual syllables. Because spectra can be influenced by the recording environment, we used songs with high signal-to-noise ratios $(\bar{x}\pm SE=43\pm 1.4 \text{ dB})$. Before spectral analyses, we removed low-frequency noise from the recordings using a finite impulse response bandpass filter (5 kHz-22 kHz, hanning window length = 512). The average spectra of each recording were normalized to probability mass functions during each discrete Fourier transform (DFT: window length = 2048 with 0% overlap), and Kolmogorov-Smirnov (K-S) distances (Gasc et al. 2013) or relative frequency dissimilarities (Deecke and Janik 2006) were computed between each pair of recordings. The K-S distances are the maximum difference between the cumulative probability mass functions of each spectrum in the pair. Relative frequency dissimilarity is a percentage based on the sum of the ratio of minimums and maximums across all frequencies in the two spectra. Only single recordings from each male were used (30 s recordings—GAL: 43, FL: 33, NC: 4Å, SC: 6Å, A. parvipennis: 5Å; single syllables—GAL: 4Å, FL: 3Å, NC: 5Å, SC: 8Å, A. parvipennis: 5Å). A hierarchical cluster analysis (hclust function in R) was performed on the distance/dissimilarity matrices to produce a dendrogram showing the relationships among the individuals' songs or syllables (Sueur 2018). To test for differences among populations, we used distance-based redundancy analysis (db-RDA, ade4 package in R) and a principal coordinate analysis (PCoA, ade4 package in R) on the distance/dissimilarity matrices with population as a factor. We then ran Monte Carlo simulations (N =10000) to test for significant clustering by population under the H_o of the db-RDA output (Sueur 2018).

Morphological measurements.-To test for differences in morphological characters among populations, we positioned preserved, pinned museum specimens so that digital images (11Mpix) could be taken of their dorsal and lateral aspects. In each photo, a scale in the same focal plane as the structures to be measured allowed calibrated measurements to be made with ImageJ software (Schneider and Rasband 2012). Measures (to the nearest 0.1 mm) included pronotal length along the midline (PrnL), maximal pronotal width (PrnW), tegminal length (TegL) and width (TegW), hindwing exposure (HwEx), femur and tibia lengths of the hindleg (FemL and TibL, respectively), and for females, ovipositor length (OviL). See also Walker et al. (2003). Measurements for each character were analyzed using ANOVA to test for differences among populations. We also used principal component analysis (PCA, ade4 package in R) on the matrix of morphological measures with population as a factor and conducted Monte Carlo simulations (N = 10000) to test for significant clustering by population under the H₂ of the PCA output.

DNA extraction and sequences.—Genomic DNA was extracted from the proximal portion of the frozen femur of individuals from field populations of purported *A. bartrami* (AL $(2 \circ 1 \circ, Cleburne Co.)$, FL $(1 \circ 1 \circ, Liberty Co.)$, GA $(1 \circ 2 \circ, Gordon Co.)$, NC $(4 \circ 2 \circ, Richmond Co.)$, *A. nr bartrami* SC $(0 \circ 1 \circ, Aiken Co.; 1 \circ 2 \circ, Edgefield Co.; 2 \circ 2 \circ, Georgetown Co.) and for$ *A. parvipennis*in $AR (<math>2 \circ 1 \circ, Faulkner Co.$) and MO $(2 \circ 1 \circ, Shannon Co.)$. We used the standard protocol for the Qiagen DNeasy tissue kit (Qiagen, Valencia, CA) and stored the gDNA extracts at either -20 °C or -80 °C until they were used for PCR amplification and sequencing.

Because reliance on a single barcoding gene might cause problems in phylogenetic analyses (Moulton et al. 2010), massively

parallel sequencing was performed to simultaneously interrogate regions of mitochondrial, and nuclear DNA for analysis. In particular, we sequenced the cytochrome oxidase subunit I (COI, 658 bp) mitochondrial gene and a large region of nuclear ribosomal DNA (rDNA) that included portions of 28S and 18S rDNA as well as the entire region of 5.8S rDNA and two internal-transcribed spacers ITS1 and ITS2 (~3700 bp). The COI gene is a barcoding gene that has short divergence times and has been used extensively in molecular systematics (Hebert et al. 2003). Ribosomal genes (28S, 5.8S, and 18S) are relatively conserved with little change over long periods of time, whereas the internal transcribed spacers are more labile and thus have been used successfully to distinguish cryptic species in some taxa (Li and Wilkerson 2005, Li et al. 2010). Additionally, we sequenced three nuclear genes, histone 3 (HIS), tubulin-alpha I (TUB), and wingless genes (WNG), that have been used in tettigoniid phylogenies (Mugleston et al. 2013).

PCR amplification.—Published primer pairs were used to amplify the regions of interest (Table 1). The Roche FastStart High Fidelity PCR System (Millipore Sigma, St. Louis, MO) was used for all amplifications. PCR amplification for the ~3700 bp rDNA region used conserved primers LR7 and NS19b with an initial denaturation at 95°C for 2 min followed by 35 cycles of 60 sec at 95°C, 60 sec at 50°C, and 5 min at 68°C plus an additional 20 seconds each successive cycle. The final PCR extension was 7 min at 72°C. PCR reactions (50 µL total volume) contained final reagent concentrations of 2.5 U of Roche FastStart High Fidelity enzyme blend, 1.8 mM MgCl₂, 0.4 µM each forward and reverse primer, 4% DMSO, and 0.2 mM each dNTP. Reverse touchdown amplification of COI used LCO1490 and HCO2198 primers and had thermal cycling parameters including an initial denaturation of 95°C for 2 min followed by 6 cycles of 30 sec at 94°C, 90 sec at 45°C, and 60 sec at 72°C and an additional 34 cycles of 30 sec at 94°C, 90 sec at 49°C and 60 sec at 72°C with a final extension of 7 min at 72°C. PCR reactions (25 µL total volume) contained final reagent concentrations of 5 U of Roche FastStart High Fidelity enzyme blend, 1.8 mM MgCl₂, 0.6 µM each forward and reverse primer, 6.25% DMSO, and 0.2 mM each dNTP. Tubulin-alpha I genes were amplified with 294F1 and 294R1 primers, histone 3 genes with H3 AF and H3 AR primers, and wingless genes with WG550F and WGABRZ primers, respectively. Tubulin-alpha I, histone 3, and wingless genes were amplified in independent PCR

reactions with thermal cycling parameters having an initial denaturation at 95 °C for 2 min followed by 35 cycles of 30 sec at 94 °C, 30 sec at 50 °C, and 50 sec at 72 °C with a final 7 min extension at 72 °C. PCR reactions (25 μ L total volume) contained final reagent concentrations of 5 U of Roche FastStart High Fidelity enzyme blend, 1.8 mM MgCl₂, 0.6 μ M each forward and reverse primer, 6.25% DMSO, and 0.2 mM each dNTP. Amplicon products were quantified using an Agilent 2100 Bioanalyzer and DNA 1000 kit (Agilent Technologies, Inc., Santa Clara, CA).

Library preparation and massively parallel sequencing (MPS).—PCR products were diluted to a concentration of 0.2 ng/µL and enzymatically fragmented and tagged with MPS sequencing adapters using the Illumina Nextera XT Library Prep kit (Illumina, Inc., San Diego, CA). Limited-cycle PCR was used to add flow cell adapters and multiplexing barcodes to fragmented libraries. Flow cell adapters enable library fragments to anchor to the surface of the solid support where sequencing occurs. Barcodes allow for postsequencing parsing of sample-dependent data, which permits a high degree of multiplexing per sequencing run. Solid-phase reversible immobilization (SPRI) beads were used to purify the prepared libraries via the removal of unincorporated primers and dNTPs that could affect sequencing downstream. Libraries were then normalized to ensure equal representation of each sample, and equal volumes were pooled to create a master library for sequencing. Sequencing was performed on an Illumina MiSeq using a v3 2 × 300 cycle kit (Illumina, Inc., San Diego, CA).

Assembly, validation, and alignment.—Sequence analyses were carried out using Geneious Prime 2020.1.2. NextGen Fastq sequences were first set as paired reads and trimmed using BBDuk with a minimum quality Q30 and a minimum length of 20. These reads were then assembled to GenBank (Clark et al. 2016) reference sequences (COI: HQ968170 and ITS/5.8s ribosomal genes: AM888963) of *Scudderia furcata*, another phaneropterine katydid, and two other sequences from members of *Amblycorypha* (tubulinalpha I: KF571404 and wingless: KU550854.1). Major vote consensus sequences were extracted from these assemblies, inspected for quality, and searched for within NCBI using BLAST (Altschul et al. 1990). All alignments were made using Clustal Omega 1.2.2 with fast clustering, a cluster size of 100, and 3 refinement iterations (Sievers et al. 2011).

Table 1. Primer pairs and annealing temperatures for PCR and expected size for sequences.

Primer	Sequence 5'3'	Anneal (°C)	%GC	Amplicon Size (bp)	Ref
COI Primers					
F LCO1490	GGTCAACAAATCATAAAGATATTGG	59.7	32.0		Folmer et al. 1994
R HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	64.5	34.6	658	Folmer et al. 1994
28S and 18S rDN	IA Primers				
F LR7	TACTACCACCAAGATCT	53.6	41.2		Vigalys and Hester 1990
R NS19b	CCGGAGAGGGAGCCTGAGAAC	68.9	66.7	~3700	Bruns Lab, UC Berkeley
Histone 3 Primer	°S				
F H3 AF	ATGGCTCGTACCAAGCAGACV	50.0	55.6		Colgan et al. 1998
R H3 AR	ATATCCTTRGGCATRATRGTG	50.0	40.5	~375	Colgan et al. 1998
wingless (wg) Pr	imers				
F WG550F	ATGCGTCAGGARTGYAARTGY	50.0	47.6		Mugleston et al. 2013
R WGABRZ	CACITNACYTCRCARCACCAR	50.0	50.0	~450	Mugleston et al. 2013
tubulin-alpha I P	rimers				
F 294F1	GAAACCRGTKGGRCACCAGTC	50.0	59.5		Buckman et al. 2012
R 294R1	GARCCCTACAAYTCYATTCT	50.0	42.5	~350	Buckman et al. 2012

Phylogenetic analysis.—Phylogenetic relationships were inferred using Bayesian analysis in *BEAST2, which uses the Markov chain Monte Carlo (MCMC) process to explore tree space based on posterior probabilities (Bouckaert et al. 2019). We used BEAUTi2 to generate the analysis parameters. We set each gene sequence as a separate partition in the multi-locus, multi-species coalescent analysis and allowed the program to integrate analytical population size. We set the site substitution model for all genes to HKY with frequencies empirically estimated. The analysis was run under a strict clock for each gene partition with priors, using the birth-death model to estimate birth and death rates during the analysis. The number of MCMC iterations was 1.2E8, which was sufficient for the model to reach stationarity after a 20% burnin. The output of each *BEAST2 run was inspected using Tracer v1.7.2., and the trees were visualized and annotated using Densi-Tree v2.2.7 and TreeAnnotator v2.6.6, respectively. TreeAnnotator produced trees with maximum clade credibility for each gene tree and for the species tree that resulted from the coalescent analysis. We used different random seeds to conduct 5 *BEAST2 analyses to ensure that the random process adequately covered tree space and that the output trees generated were robust. We ran the analyses with all populations separated and with putative 'species' that



were suspected based on differences in syllable rate functions with temperature (see below).

Deposition of specimens, recordings, and sequences.—Unless otherwise indicated, the specimens are currently housed at the University of North Carolina at Asheville (UNCA). The collection, along with types, will be transferred to the Florida State Collection of Arthropods (FSCA), Gainesville, FL. Recordings will be made available through the Macaulay Library of Natural Sounds at the Cornell Lab of Ornithology and Singing Insects of North America (SINA) website. Sequencing data have been uploaded to the National Center for Biotechnology Information (NCBI) under BioProject PRJNA906584.

Results

Song variation.—Figs 1–6 show the temporal structure of calling songs among populations. For all populations, the calling songs are a series of easily quantified, repeated syllables representing a single cycle of wing movement. The calling songs of *A. bartrami* and *A. parvipennis* do not exhibit the extreme song complexity of the virtuoso *Amblycorypha*, which have 4 syllables



Fig. 1. A–C. Oscillograms (30s) of calling songs of 3 male *A. bartrami* from Liberty Co., Florida. Songs consist of a long duration, sustained main series of (~100) syllables preceded by several (15–20) short-duration series of 1–7 syllables; **D.** Oscillogram showing 16 syllables within yellow highlighted portion of the main series of **C**; **E.** Oscillogram and spectrogram showing the fine temporal structure and frequency content of 3 syllables highlighted in **D**.

Fig. 2. A–C. Oscillograms (30s) of calling songs of 3 male *A. nr* bartrami from Aiken Co., South Carolina. Songs consist of a long duration, sustained main series of syllables preceded by several shortduration series of 1–5 syllables; **D.** Oscillogram of 15 syllables highlighted in **C**; **E.** Oscillogram and spectrogram showing the fine temporal structure and frequency content of 3 syllables highlighted in **D**.

that may be produced with varying syntaxes (Walker and Dew 1972, Walker 2004). In most cases, the songs of *A. bartrami* consist of a longer duration, sustained (main) series of syllables preceded by 1 to >20 shorter series that typically increase in amplitude. Calling songs vary significantly among the populations in several ways.

Temporal variation.—Songs of males from the Florida panhandle (N=33: 9 series) have sustained portions with significantly more syllables ($\bar{x}\pm$ SE=107 \pm 22) than all other populations (GAL: 8 \pm 1, N=43: 53 series; NC: 17 \pm 1, N=83: 30 series; SC: 25 \pm 2, N=113: 112 series; *A. parvipennis*: 24 \pm 3 N=63: 39 series; Fig. 7). Males from NC nearly always produced syllables in doublets during the sustained main portion of their calling song (Fig. 5E). Of the 517 syllables produced in 30 main series from 8 males, 482 were doublets, 8 (2%) were singlets, and 9 (5%) were triplet syllables.

Series that precede the main series of calling songs have, on average, 5–6 syllables for males from FL, whereas those in songs of males from other populations have fewer (GAL: 3–8; NC: 2–3; SC: 2–4; *A. parvipennis*: 1–2; Fig. 7). Males of *A. parvipennis* rarely (13%, 5 of the 39 series from 6 males) produce syllables preceding the main series of their calling songs (Fig. 7). See Suppl. material 3.

Syllable rate variation.—Based on the relationships of syllable rates with temperature (Fig. 8, Suppl. material 2), there are at least 4 different song types across the populations we sampled. Males from northern GA and northern AL (GAL) have functions with the greatest slopes (m=0.81, Fig. 8: green) and rates of ~13.1s⁻¹ at 25°C. SC males (A. nr bartrami) have the slowest syllable rates at ~5.8s⁻¹ at 25°C (m=0.29, Fig. 8: orange), which is very similar to that of A. parvipennis ~5.0s⁻¹ at 25°C (m=0.16, Fig. 8: black). Males from northern FL and the FL panhandle produce syllables at intermediate rates of 10.0s⁻¹ at 25°C (Fig. 8: red). Males from western AL had songs with syllable rates similar to those of FL males (Fig. 8: pink). Because NC males produced songs with syllable doublets, two rates were calculated. The faster syllable rate, within a doublet (m=0.58, 11.6s⁻¹ at 25°C), falls between rates for songs of FL males and those of GA and AL males whereas the slower rate, between doublets (m=0.17, \sim 4.0s⁻¹ at 25°C), was slower than the rates of SC A. nr bartrami and A. parvipennis males.

Syllable variation.—Fig. 9 shows the variation in the fine temporal structure of syllables produced by males from each population. The impulses in each syllable are probably the result of the scraper engaging and releasing a single tooth on the file. Males from FL have a single pulse train followed by a longer



Fig. 3. A–C. Oscillograms (30s) of calling songs of 1 male *A. bar-trami* from Gordon Co., Georgia. The long-duration sustained, main series of syllables are rarely preceded by shorter series as found in the songs from other supposed populations of *A. bar-trami*; **D.** The yellow highlighted portion of **C**; **E.** Oscillogram and spectrogram of 3 syllables highlighted in **D**.

Fig. 4. A–C. Oscillograms (30s) of calling songs of 3 male *A. bartrami* from Cleburne Co., Alabama. Syllable rates of sustained main series are similar to those of males from north Georgia (Figs 3, 7); **D.** Oscillogram of 17 syllables highlighted in C; **E.** Yellow highlighted portion of **D** showing detailed temporal structure and spectral composition of 3 syllables.



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Fig. 5. A–C. Time waveforms (30s) of calling songs for 3 NC *A. bartrami* males. Note the variation in the number of syllables that precede the main sustained train of syllables; **D.** Highlighted portion in **C**; **E**. Oscillogram and spectrogram of highlighted portion of **D** with 6 syllables in 3 doublets. Doublet syllable rates were faster than those of FL *A. bartrami* and slower than those of GA-AL *A. bartrami*. FL, GA, AL, and SC males rarely produced doublet syllables.

terminal pulse train in the syllable. In all other populations, males exhibited two pulse trains made up of 1–5 pulses before the terminal pulse train.

Spectral variation.-The signals produced by males are broadband, with most of the energy between 10-15 kHz (Panel E of Figs 1-6). Hierarchical cluster analyses using K-S distance and relative frequency dissimilarity metrics indicate that significant spectral variation exists between populations of A. bartrami, A. nr bartrami, and A. parvipennis at the level of calling song and syllables (Figs 10, 11, respectively, Suppl. material 4). Although there is much overlap among populations in the dendrograms (Figs 10A, B, 11A, B), principal coordinate analyses calculated on the distance/dissimilarities show significant clustering within populations and differences among populations. Average spectra computed over the entire calling song (30s) differed significantly from random when using population as a factor for both distance metrics (K-S distance, Monte-Carlo test simulation, N=10000, p=0.032, first two components explain 77% of total variance; relative frequency dissimilarity, Monte-Carlo test simulation, N=10000, p=0.018, first two components explain 43% of total variance; Figs 10C, D). The same was found when the average spectra were calculated on smaller time scales associated with single syllables (K-S distance, Monte-Carlo test simulation, N=10000, p<0.008,

Fig. 6. A–C. Oscillograms (30s) of calling songs of 3 male *A. parvipennis*; **D.** Oscillogram of 10 syllables highlighted in **C**; **E.** Fine temporal structure and spectrogram of 3 syllables highlighted in **D**.

first two components explain 74% of total variance; relative frequency dissimilarity, Monte-Carlo test simulation, N=10000, p<0.002, first two components explain 32% of total variance; Figs 11C, D).

Morphological variation.—Morphological characters differed among some of the populations (Table 2, Suppl. material 1). The only significant differences among females' character measurements were pronotal length (PrnL), where GAL females have significantly shorter PrnL (5.99 ± 0.23 mm) than SC females (6.60 ± 0.07 mm), and respective *A. parvipennis* females (6.96 ± 0.19 mm). The lack of significant differences among any other female morphological measurements may, in part, be due to the small sample sizes associated with many of the populations.

There were many differences in male size among some of the populations. Similar to our findings for female PrnL, GAL males also had significantly shorter PrnL (5.08±0.14 mm) than SC males (6.03±0.09 mm) and *A. parvipennis* males (6.15±0.13 mm). For nearly every morphological measurement (TegL, TegW, FemL, TibL), GAL and *A. parvipennis* were shorter than the other populations (Table 2). GAL males differed significantly from NC males in all measures except HwEx (PrnW: 3.64±0.08 vs 4.32±0.06 mm, respectively; TegL: 25.4±0.70 vs 28.2±0.21 mm, respectively; TegW: 7.83±0.21 vs 9.32±0.18 mm, respectively; FemL: 23.3±0.11 vs 27.3±0.38 mm, respectively; TibL: 25.1±0.20 vs 28.7±0.44 mm, respectively). GAL males had significantly shorter hind femurs (FemL) and hind tibiae (TibL) than that of all other purported *A. bartrami* populations (Table 2).



Fig. 7. Mean (±SE) number of syllables as a function of the temporal relationship between series in male calling song from populations of supposed *Amblycorypha bartrami* (GA & AL: green, NC: blue, FL: red, SC: orange) and *A. parvipennis* (black). Triangles (X=0) represent the mean number of syllables in the sustained main series of songs averaged over the number of males shown in parentheses. Circles are the means for each series preceding the main series with the number of males contributing to the average indicated in parentheses.



Fig. 8. Syllable rate as a function of temperature for populations of *Amblycorypha bartrami* (GA & AL: green, NC: blue, FL: red, AL: pink, SC: orange) and *A. parvipennis* (black). Solid symbols are recordings from our research and open symbols are recordings from other published work (Walker et al. 2003 for *A. bartrami* and *A. parvipennis*, Shaw et al. 1990 for *A. parvipennis*).

Because the functions of syllable rate and temperature between SC *A. nr bartrami* and *A. parvipennis* are so similar, it is important to compare the morphological traits among them. Male SC *nr bartrami* had significantly longer tegmina (TegL: 26.2 ± 0.44 vs 23.8 ± 0.47 mm, respectively) and significantly longer hindwing exposure (HwEx: 2.74 ± 0.17 vs 0.89 ± 0.34 mm, respectively) than *A. parvipennis* males. Hindwing exposure is one of the key characteristics distinguishing *A. parvipennis* from all eastern members of the *rotundifolia* complex (Rehn and Hebard 1914).

Principal component analysis indicates morphological differences among the supposed populations of *A. bartrami* (Fig. 12). In



Fig. 9. Syllable variation among populations of *A. bartrami*. A. Florida N=33; B. Alabama N=33; C. Georgia N=13; D. South Carolina N=53; E. North Carolina N=53. Syllables consist of brief decaying impulses that are likely the result of the scraper engaging and releasing a single tooth on the file. Males from FL (A.) typically have a single pulse train of 1–2 pulses preceding the longer terminal pulse train in the syllable. Males from all other populations have two pulse trains (1–5 pulses) preceding the terminal pulse train. Scale bars: 100ms.

particular, the slope of the ordination in the PCA for GAL males is negative, whereas it is positive for all other populations whose relationships are all parallel (Fig. 12). FL, NC, and SC males tended to be more similar in the averages of their morphological characters. Characters that contributed most to PC1 were TibL (20%), FemL (19%), PrnW (16%), TegL (16%), and TegW (16%), and those that contributed most to PC2 were HwEx (40%), PrnL (28%), and TegL (14%).

Genetic variation.—Once sequences were processed and aligned, the lengths of consensus sequences were COI: 658 bp, H3: 333 bp, ITS1, ITS2, and 5.8S ribosomal genes = ITS3k: 3262 bp, TUB: 341 bp, and WNG: 371 bp. BLAST searches of each gene sequence, except for TUB, invariably matched those of *Amblycorypha* or other members of the Phaneropterinae (COI: all individuals >90% match to COI of *Amblycorypha floridana* [HQ983647.1, HQ983648.1, HQ983649.1], *Amblycorypha oblongifolia* [HQ983655.1, JN294610.1, KM532357.1, KM536809.1, KR144595.1] or *Amblycorypha* sp. [MG466233.1]; H3: all individuals 100% match to histone 3 gene of *Amblycorypha* sp. [KF571154.1]; ITS3k: all individuals >98% match to 28S *Microcentrum rhombifolium* or *Scudderia furcata;* WNG: all individuals

Sex Population	PrnL	PrnW	TegL	TegW	FemL	TibL	OviL
Females							
FL	6.52 ^{ab}	4.33ª	28.6	9.11	29.6	30.6	9.83
	(NA, 1)	(NA, 1)					
GAL	5.99 ^b	3.86ª	26.0	8.07	25.9	26.6	10.7
	(0.23, 5)	(0.13, 5)	(0.67, 5)	(0.34, 5)	(0.49, 5)	(0.59, 5)	(0.38, 5)
NC	7.05 ^{ab}	4.58ª	28.0	9.63	29.9	31.0	11.0
	(NA, 1)	(NA, 1)					
SC	6.60ª	4.12ª	26.6	8.12	27.6	28.7	9.93
	(0.07, 17)	(0.08, 17)	(0.47, 13)	(0.25, 15)	(0.40, 15)	(0.40, 14)	(0.25, 17)
A. par	6.96ª	4.52ª	25.8	7.81	27.1	28.3	9.9
	(0.19, 3)	(0.07, 3)	(0.78, 3)	(0.27, 3)	(0.54, 3)	(0.68, 3)	(0.54, 2)
Males							
FL	5.88 ^{ab}	4.01 ^{ab}	29.7ª	9.31 ^{ab}	28.0ª	28.8ª	
	(0.05, 3)	(0.14, 3)	(0.72, 3)	(0.17, 3)	(0.38, 3)	(0.59, 3)	
GAL	5.08 ^b	3.64 ^b	25.4 ^{cd}	7.83 ^b	23.3 ^c	25.1°	-
	(0.14, 7)	(0.08, 7)	(0.70, 7)	(0.21, 7)	(0.11, 7)	(0.20, 7)	
NC	5.84 ^{ab}	4.32ª	28.2 ^{ab}	9.32ª	27.3 ^{ab}	28.7 ^{ab}	-
	(0.04, 9)	(0.06, 9)	(0.21, 9)	(0.18, 9)	(0.38, 6)	(0.44, 6)	
SC	6.03ª	4.00 ^{ab}	26.2 ^{bc}	8.21 ^b	27.1 ^{ab}	28.2 ^{ab}	-
	(0.09, 17)	(0.09, 17)	(0.44, 17)	(0.17, 17)	(0.42, 15)	(0.34, 15)	
A. par	6.15ª	3.97 ^{ab}	23.8 ^d	7.71 ^b	25.5 ^{bc}	26.6 ^{bc}	-
	(0.13, 8)	(0.08, 8)	(0.47, 8)	(0.25, 8)	(0.48, 8)	(0.41, 8)	

Table 2. Mean (SE, N) of morphological measures (mm) from populations of supposed *Amblycorypha bartrami* and populations of *A. parvipennis.**

* Comparisons of means within each sex were made for each morphological trait. Means within a column followed by different letters are significantly different (ANOVA, Tukey honest significant difference posthoc test, P<0.05).

>99% match to WNG *Amblycorypha longinicta* [KU550854.1] or *Amblycorypha* sp. [KF571288.1]. There was no genetic variation in H3 among all samples; therefore, we did not include H3 sequences in any further analyses. For all individuals sequenced, TUB sequences matched tubulin-alpha I sequences of members in the Tettigoniidae, with 11 individuals matching (85–97% identical) sequences in the Phanertopterinae (*Syntechna* and *Trigonocorypha*), 11 individuals matching (79–80% identical) *Lipotactes maculatus* (Lipotactinae), and one individual matching 82% of the tubulin-alpha I sequence of *Kuzicus megaterminatus* (Meconematinae). Because these matches for TUB were so varied, we ran the *BEAST2 analyses with and without TUB sequences included.

Multiple runs of our multispecies coalescent analyses produced identical phylogenetic topologies with only small differences in the posterior probabilities at the nodes. Effective sample sizes (ESS) for every parameter of the models were always over 1500.

Gene trees.—Gene trees based on COI, ITS3k, and WNG sequences were similar to the species trees generated in our analysis (Figs 13, 14). Gene trees using TUB sequences differed substantially from the species trees. The estimated mutation rate of mitochondrial gene COI was about 45X that estimated for ITS3k, about 10X that for TUB, and almost 15X that for WNG.

Our gene tree for COI using the Bayesian coalescent approach showed high support for most populations (AL, GA, NC, SCG, AR *A. parvipennis*, MO *A. parvipennis* with all posterior probabilities >0.97, Fig. 13A). The greatest uncertainty involved the two South Carolina populations (SCA and SCE) where we had data from only single individuals. There was high support for grouping the South Carolina population (Georgetown Co., SCG) with the North Carolina population (posterior probability = 1.0).

Support values for our ribosomal gene trees were variable. The tree supported monophyly of *A. parvipennis* (posterior = 1.0),

grouped the two individuals from South Carolina together (SCA and SCE, posterior probability = 0.98), grouped two of the SCG individuals with all North Carolina individuals (posterior probability = 1.0), grouped all GA individuals with most of the AL individuals, and grouped the two FL individuals (posterior probability = 0.79) (Fig. 13B).

The gene trees for our nuclear sequences (TUB and WNG) differed more from the population/species trees than the COI and ITS3k gene trees. Interestingly, the *A. parvipennis* populations clustered in the middle of both gene trees (Fig. 13C, D).

Species/population trees.—The output of our coalescent analyses (trees with maximum clade credibility) indicated genetic divergences among all populations that we sampled (Fig. 14A). Our phylogenetic analyses showed that AR and MO populations of A. parvipennis differed genetically and are more closely related to each other than they are to all supposed A. bartrami populations we sampled (posterior probability = 1.0). The population tree indicates (Fig. 14A) that A. bartrami populations from AL and FL split more recently and that there was an earlier divergence from populations in GA. Populations of supposed A. bartrami in the west (AL, FL, and GA) differ from those in the east (NC and SC). Interestingly, populations from within SC differ from each other genetically although they have identical syllable rates in their songs. South Carolina A. nr. bartrami from Georgetown Co., SC were found to be more closely related to NC 'bartrami' than to populations in Edgefield Co. or Aiken Co., SC A. nr. bartrami. Note that the NC and Georgetown Co. SC populations are also closer geographically (see *Phylogeography* below).

When the analysis was done with individuals grouped by calling song information (Fig. 14B), song morphs from GAL (GA and AL) diverged from those in FL and differed (posterior probability = 1.0) from the two eastern song morphs NC and SC (posterior



Fig. 10. A, B. Song Dendrograms. The hierarchical cluster analyses are based on average spectra of 30s recordings of individual calling songs of males from populations of *A. bartrami* and *A. parvipennis*; **C, D.** Principal Coordinate Analyses calculated on distance/dissimilarity matrices of each pair of average spectra of the same 30s recordings in **A, B**. Shaded ellipses encompass 95% of observations expected for populations (green: GAL: 4Å; red: FL: 3Å; blue: NC: 4Å; orange: SC: 6Å; black: *A. parvipennis*: 5Å). Clustering by populations differed significantly from H_o (no relationships between ordination axes) for **C** (Kolmogorov-Smirnov Distance, Monte-Carlo test simulation, N=10000, p=0.032, first two components explain 77% of total variance) and **D** (Relative Frequency Dissimilarity, Monte-Carlo test simulation, N=10000, p=0.018, first two components explain 43% of total variance).

probability = 0.77). Data that include genetic information from other members of the *rotundifolia* complex indicate that the purported populations of *A. bartrami* we studied are not monophyletic, as suggested in the phylogenies we present (Forrest unpublished, Sither 2018).

Phylogeography.—The phylogeographic relationships among the populations we studied indicate that proximity of populations is related to the genetic distances among them (Fig. 15). The two populations of *A. parvipennis* were clearly isolated genetically and geographically from the eastern populations we studied. Although individuals from AL populations were closely related to FL populations (Fig. 15A), when the phylogenetic analysis was done fac-

toring in song rate, GA and AL populations coalesced and differed from FL populations (Fig. 15B). Similarly, SCG populations from the coast (Georgetown Co.) clustered with nearby NC populations, whereas they clustered with the other SC counties (SCA, SCE) when taking song rates into account to delineate species in the coalescent model.

Discussion

Amblycorypha in North America have been assigned to three species groups based on morphology (Rehn and Hebard 1914, Walker et al. 2003, Walker 2004). Those in the *oblongifolia* complex are relatively large, those in the *uhleri* group are small, and those in



Fig. 11. A, B. Syllable Dendrograms. The hierarchical cluster analyses are based on average spectra of pairs of syllables in male songs from populations of *A. bartrami* and *A. parvipennis*; **C, D.** Principal Coordinate Analyses calculated on distance/dissimilarity matrices of each pair of average spectra of the same recordings in **A**, **B**. Shaded ellipses encompass 95% of observations expected for populations (green: GAL: 4♂; red: FL: 3♂; blue: NC: 5♂; orange: SC: 8♂; black: *A. parvipennis*: 6♂). Clustering by populations differed significantly from H_o (no relationships between ordination axes) for **C** (Kolmogorov-Smirnov Distance, Monte-Carlo test simulation, N=10000, p<0.008, first two components explain 74% of total variance) and **D** (Relative Frequency Dissimilarity, Monte-Carlo test simulation, N=10000, P=0.002, first two components explain 32% of total variance)).

the *rotundifolia* complex are intermediate in size. Members of the *uhleri* and *oblongifolia* groups can fly, whereas the medium-sized members of the *rotundifolia* group are flightless and individuals move only about 10–15 m per day (Shaw et al. 1981, Cusick 2008).

The populations we studied in this paper are in the *rotundifolia* complex and were originally considered members of *A. bartrami* because of similarities in their calling song (consisting of a series of single syllables with short bouts of syllables leading up to a longer sustained series with a rate of about 10–13 syllables per sec), their morphology, and their habitat (longleaf pine, turkey oak sandhills). Our analyses show that there are significant differences in calling songs, morphology, and genetics between some of these populations.

Song variation.—The songs of *A. bartrami*, *A. nr bartrami*, and *A. parvipennis* are relatively simple and have only a single syllable type. They do not exhibit the extreme song complexity of the virtuoso katydids (Walker et al. 2004), which have 4 syllable types usually produced in sequence but can be quite varied in their order, for example, *A. longinicta* (Walker 2004). In general, phaneropterine songs are extremely diverse with a wide range of complexity that has evolved multiple times (Heller et al. 2015), probably in response to duetting courtship signals and the evolution of countermeasures to eavesdropping by rival males (Heller et al. 2017). Closely related species may have simple songs with a single syllable type, while others have multiple syllables with varying syntax (Heller et al. 2015, ter Hofstede et al. 2020).



Fig. 12. Principal Components Analysis on six morphological measures of males from supposed populations of *A. bartrami* and populations of *A. parvipennis*. (green: GAL: 73; red: FL: 33; blue: NC: 63; orange: SC: 133; black: *A. parvipennis*: 83). Shaded ellipses around the means encompass 95% of the expected values for each population. The first two dimensions of the analysis account for 84% of the variation among the morphological measures and clustering of populations showed significant relationships in the ordination of the two dimensions (Monte-Carlo test simulation, N=10000, p<0.0001).

Among cryptic species complexes in the phaneropterines, changes in syllable rates as a function of temperature are often used to recognize species (Walker et al. 2003, Dutta et al. 2017, Heller et al. 2017). In our analysis of the temporal features of the calling songs of supposed members of A. bartrami, we found populations whose calls differed in terms of the functions of syllable rate in response to changes in temperature (Fig. 8), differed in the number of syllables and their fine temporal structure (Figs 7, 9, respectively), and differed in the overall call structure (Figs 1-6). Using these temporal differences in calling songs as a clue, we combined data from populations having similar syllable rate functions and analyzed the spectral features of their song and their morphology. Cluster analyses of the spectral features of songs (Figs 10, 11) and morphological measures (Fig. 12) showed significant grouping among the populations we studied. In addition, our phylogenetic analysis using multiple loci in a multispecies coalescent model showed genetic divergence among all populations (Figs 13, 14), suggesting spatial structure and isolation among them (Fig. 15).

Isolation and population spatial structure.—Spatially structured populations may be caused by variation and heterogeneity in the landscape and depend on adaptation to the local environment and variation in the strength of gene flow across that landscape (Revilla and Wiegand 2008, Rettelbach et al. 2016, Pina and Schertzer 2018). Longleaf pine turkey oak sandhills are a stable, fire-adapted fire-climax community (Croker and Boyer 1975, Peet and Allard 1993). One might expect that loss of flight (as in *A. bartrami*) would evolve in stable habitats compared with



Fig. 13. Gene trees based on Bayesian multi-locus coalescent analyses. Numbers in branches are the Bayesian posterior probabilities from the analysis. Colored rectangles represent song morphs identified by relationships of syllable rate and temperature (green: GAL; red: FL; blue: NC; orange: SC; black: *A. parvipennis*). **A.** Cytochrome Oxidase I, barcoding gene (COI: 658bp); **B.** ITS1, ITS2, and 5.8S ribosomal genes (ITS3k: 3262bp). **C.** Tubulin-alpha I nuclear gene (TUB: 341bp). **D.** Wingless nuclear gene (WNG: 371bp). Phylogenies were plotted using R package ggtree (Yu et al. 2017).



Fig. 14. Phylogenetic relationships (population/species trees) based on multi-locus coalescent analyses for populations of *A. bartrami* and *A. parvipennis*. Branch numbers are the Bayesian posterior probabilities for members in that lineage. Time shown is substitutions per site. Both topologies are robust across several analyses with different random starting points. A. Analysis for all populations sampled; B. Analysis for putative species (song morphs) based on relationships of syllable rate with temperature. Phylogenies were plotted using R package ggtree (Yu et al. 2017).

higher vagility adapted to more ephemeral and unpredictable environments (Grzywacz et al. 2018). Many ancient and present-day river drainages flowing from the Appalachian Mountains to the Atlantic Ocean and the Gulf of Mexico have, and continue to, fragment the longleaf pine ecosystem. River systems and their hydrogeological history have influenced isolation and speciation in fish (Hocutt et al. 1986, Mayden 1988), frogs (Lemmon et al. 2007), and salamanders (Kozak et al. 2006, Kuchta et al. 2016) of the Atlantic Slope and the Appalachian Mountains. Additionally, the longleaf pine ecosystem has experienced considerable fragmentation due to anthropogenic habitat loss and degradation (Peet and Allard 1993). Given the flightless behavior of the katydids we studied and the fragmentation of the Atlantic Slope by rivers and anthropogenic change, gene flow among populations is probably reduced. Reduced gene flow contributes to a complex spatial structure among populations and provides opportunities for local genetic changes through drift or selection that might lead to divergence and speciation (Fig. 15). Interestingly, A. arenicola, a member of the flight-capable uhleri complex, co-occurs with A. bartrami in the sandhill populations we sampled in north Florida and North Carolina. While the populations we studied were found to differ genetically across that ~700 km distance, A. arenicola does not (unpublished data).

Genetic variation: Gene trees vs species trees.—Discordance between gene trees and species trees can be caused by various evolutionary processes, including incomplete lineage sorting, gene duplication, hybridization, and gene flow (Mallo and Posada 2016). We used multiple genes and a multispecies coalescent analysis to account for incomplete lineage sorting, and here we discuss the gene trees from our analysis to consider the potential problems with each in determining the species/population trees.

Nuclear mitochondrial pseudogenes (numts) may be coamplified with COI. These pseudogenes are difficult to detect and may influence barcoding results. Mitochondrial pseudogenes occur in a wide diversity of Orthoptera. Hawlitschek et al. (2017) showed that only 76% of the orthopteran species they studied were reliably identified by barcoding genes and mostly agreed with traditional taxonomy. However, some DNA barcoding sequences had large genetic distances within a species and,



Fig. 15. Phylogeography of 'A. *bartrami*'. Phylogenies were determined by a Bayesian multi-locus multi-species coalescent model using a Markov Chain Monte Carlo process. A. Phylogeographic distribution of sampled populations of supposed A. *bartrami* (AL, FL, GA, NC, and SC) and A. *parvipennis*; B. Phylogeographic distribution of song morphs that differed in syllable rates as a function of temperature. Plots were constructed using plot.to.map function of the R package phytools (Revell 2012).

in some cases, had identical DNA haplotypes between morphologically/ecologically divergent species, which were most likely caused by incomplete lineage sorting or hybridization (Hawlitschek et al. 2017). Using only DNA barcoding genes, genealogical paraphyly is common in many groups of closely related animals (see Trewick 2008). Research using only COI to determine relationships among closely related phaneropterines has not always confirmed relationships and species predicted from morphology, acoustic signals or ecology (e.g. Kensinger et al. 2017, Kocinski 2020). Our gene tree for COI using the Bayesian coalescent approach showed high support for most populations and, in general, agreed with data from our behavioral (acoustic signals) and morphological analyses. One COI sequence from an individual from Edgefield Co., SC was very different from the other A. nr bartrami (SCE, Fig. 13A), even though the BLAST search of this sequence closely matched Amblycorypha floridana and Amblycorypha oblongifolia sequences (HQ983649.1 and HQ983655.1, respectively).

In addition to COI, we sequenced the 5.8S ribosomal gene and internal transcribed spacers ITS1 and ITS2. ITS1 and ITS2 sequences have been useful in finding species-level differences in some insect groups, including katydids. Ullrich et al. (2010) investigated ITS1 and ITS2 from barbistine katydids and tested whether the secondary structure, which is determined by the interdependency of the interacting nucleotides of these ribosomal genes, might be useful in phylogenetic analyses. They found that ITS2 had two secondary structures similar to those known from other eukaryotes. ITS1 was much more variable, and it was, therefore, more difficult to predict its secondary structure. We did not investigate the secondary structure of the ITS DNA sequences. The 5.8S and ITS sequences appeared to improve our phylogenetic analysis and grouped AL and GA populations as well as NC with some SC populations.

Tubulin-alpha I genes have evolved through gene duplication in insects, and paralogues of tubulin may have different sequences (Nielsen et al. 2010) that could have influenced our gene tree. The information from our TUB sequences did not sort populations and song morphs in ways that we expected. Interestingly, eliminating TUB sequences from the analysis did not change the topology of the resulting species trees. Indeed, support values for grouping song morphs were greater when TUB sequences were included in the coalescent analysis. The WNG gene tree, although having many branches with low support values, consistently grouped populations of *A. parvipennis* and grouped individuals from the song morphs (GA with AL populations, NC populations, and SC populations).

Including nuclear, ribosomal, and mitochondrial genes in our analysis probably improved our overall understanding of the relationships among the populations we studied. Our results were similar to those of Kim et al. (2016), who found lower divergence in nuclear genes (<1% divergence) compared with mitochondrial genes (3–7% divergence) in *Tettigonia* from South Korea. Adding nuclear genes to their analysis contributed to an improved phylogenetic signal, which aided in identifying cryptic species.

Songs and diversity.—In phaneropterines, speciation may begin as the result of sexually selected changes occurring in the song structure of local populations that cause divergence (Heller et al. 2015). Because duets play important roles in phaneropterine courtship and allow for eavesdropping by individuals outside the duet, song complexity has probably evolved as countermeasures to eavesdropping (Villarreal and Gilbert 2014, Heller et al. 2015, Heller et al. 2017, Heller and Hemp 2020). Heller et al. (2017) found cryp-

tic ethospecies in *Ducetia japonica* (long-winged and widespread) that showed little or no difference in genitalic diversity but had very different songs, and the file teeth associated with the stridulatory apparatus differed in shape, number, and size.

Small changes in timing and temporal song structure are enough to cause behavioral isolation in phaneropterines. Female *Mecopoda elongata* from populations that 'chirp' distinguish between trilling and double chirp songs (Dutta et al. 2017). The double chirpers of *M. elongata* have higher song rates. Similarly, we found that NC males produce doublet syllables with higher rates compared with their closely related geographic neighbors in SC.

Flightlessness, which probably decreases gene flow, may increase the rates of divergence among populations. Hemp et al. (2009) found that flightless *Monticolaria* katydids in Africa were isolated on mountain ranges, resulting in speciation. We believe that river systems have probably isolated populations of flightless members of the *rotundifolia* complex inhabiting the coastal plain of the southeastern United States and that this isolation allowed for divergences in their song, morphology, and genetics.

Taxonomy.—Based on our work and the differences in song, morphology, and genetics that we found among the populations we studied, we describe three new species of round-winged katydids: *A. carolina* sp. nov., *A. peedee* sp. nov., and A. *tallapoosa* sp. nov.

Tettigoniidae Krauss, 1902 Phaneropterinae Burmeister, 1838 Amblycoryphini Brunner von Wattenwyl, 1878

Amblycorypha Stål, 1873

Type species.—*Amblycorypha oblongifolia* (De Geer, 1773).

Amblycorypha carolina Spooner & Forrest, sp. nov. https://zoobank.org/A279436F-23BC-492F-B8C6-9EA7A6E7E430 Figs 2, 7–16, Table 2

Material examined.— Holotype: USA ● ♂; South Carolina, Georgetown, Hobcaw Barony, Kings Rd; 33.3480°N, 79.227°W; 5 Jun. 2009; T.G. Forrest and L.D. Block leg.; Anb-M04-2009; DNA (MS-034) NCBI accession SAMN31929333; REC (2009 Tape02 PGM 1023); UNCA to be transferred to FSCA.

Allotype: USA • \bigcirc ; South Carolina, Georgetown, Hobcaw Barony, Kings Rd; 33.3480°N, 79.2271°W; 5 Jun. 2009; T.G. Forrest and L.D. Block leg.; Anb-F03-2009; DNA (MS-004) NCBI accession SAMN31929331; REC (2009 Tape02 PGM 1024 duet with M06), REC (2009 Tape02 PGM 1025 duet with M02); UNCA to be transferred to FSCA.

Paratypes: (163, 169) USA • 19, 13; South Carolina, Aiken Co; 14 May 2008; J.D. Spooner leg.; UNCA to be transferred to FSCA • 19; South Carolina, Aiken Co; 24 May 2008; J.D. Spooner leg.; UNCA to be transferred to FSCA • 19, 13; South Carolina, Aiken Co; 30 May 2008; J.D. Spooner leg.; UNCA to be transferred to FSCA • 29, 33; South Carolina, Aiken Co; 4 Jun 2008; J.D. Spooner leg.; UNCA to be transferred to FSCA • 13; South Carolina, Aiken Co; 27 May 2019; T.G. Forrest leg.; UNCA to be transferred to FSCA • 29, 13; South Carolina, Edgefield Co; 21 May 2008; J.D. Spooner leg.; UNCA to be transferred to FSCA • 29, 13; South Carolina, Edgefield Co; 21 May 2008; J.D. Spooner leg.; UNCA to be transferred to FSCA • 33; South Carolina, Edgefield Co; 24 May 2010; J.D. Spooner leg.; UNCA to be transferred to FSCA • 33; South Carolina, Edgefield Co; 5 Jun.



Fig. 16. Holotype of *Amblycorypha carolina*, Carolina round-winged katydid. A. Dorsal view; B. Lateral view. Scale bars: 5 mm.

2007; J.D. Spooner leg.; UNCA to be transferred to FSCA • 3°_{\uparrow} , 5°_{\uparrow} ; South Carolina, Georgetown Co; 5 Jun. 2009; T.G. Forrest and L.D. Block leg.; UNCA to be transferred to FSCA.

Other specimens.—43, 49 from Walker et al. 2003: USA • 23; South Carolina, Aiken Co; 17 Jun. 68; J.D. Spooner leg.; FSCA • 19; South Carolina, Aiken Co; 10 Jul. 87; J.D. Spooner leg.; FSCA • 13; South Carolina, Aiken Co; 7 Jun. 88; J.D. Spooner leg.; FSCA • 13; South Carolina, Aiken Co; 21 Jun. 88; J.D. Spooner leg.; FSCA • 19; South Carolina, Aiken Co; 14 Jun. 93; J.D. Spooner leg.; FSCA • 19; South Carolina, Aiken Co; 21 Jun. 93; J.D. Spooner leg.; FSCA • 19; South Carolina, Aiken Co; 21 Jun. 93; J.D. Spooner leg.; FSCA • 19; South Carolina, Edgefield Co; 7 Jun. 88; J.D. Spooner leg.; FSCA.

Size measurements (mm).—Holotype: PrnL: 6.1, PrnW: 4.4, TegL: 28.0, TegW: 8.9, HwEx: 2.2, FemL: 28.2, and TibL: 29.7 mm (Fig. 16). Allotype: PrnL: 6.7, PrnW: 4.4, TegL: 26.4, TegW: 7.5, HwEx: NA, FemL: 28.9, TibL: 29.0, OviL: 8.8.

Etymology.—This species is named for its geographic location within South Carolina, north of the Savannah River and south of the Pee Dee River.

Common name.-Carolina round-winged katydid.

Differential diagnosis.—Members of this species are best distinguished from other members of the *rotundifolia* species group and from *A. bartrami*, in particular by calling song. Syllable rates as a function of temperature are ~5.8s⁻¹ at 25 °C (Fig. 8) and differ from all other eastern members of the *rotundifolia* complex. Although the syllable rates of *A. carolina* are similar to *A. parvipennis* from the western USA, *A. carolina* have significantly longer tegminal lengths and hindwing exposure (Table 2). Calling songs of *A. carolina* have a sustained series of about 25 syllables compared to more than 100 syllables in the sustained portion of songs from Florida *A. bartrami* (Fig. 7). Series that preceded the main, sustained series also tend to have fewer syllables than Florida *A. bartrami* (2–4 vs 5–6, respectively Fig. 7).

Description.—Individuals are typically green and have all attributes of members of the *rotundifolia* complex of *Amblycorypha* (Walker et al. 2003). Female size measurements ($\bar{x}\pm$ SE in mm, N) are on average PrnL: 6.60±0.07, 17; PrnW: 4.12±0.08, 17; TegL: 26.6±0.47, 13; TegW: 8.12±0.25, 15; FemL: 27.6±0.40, 15; TibL: 28.7±0.40, 14; OviL: 9.93±0.25, 17. Males size measurements are on average PrnL: 6.03±0.09, 17; PrnW: 4.00±0.09, 17; TegL: 26.2±0.44, 17; TegW: 8.21±0.17, 17; FemL: 27.1±0.42, 15; TibL: 28.2±0.34, 15 (Table 2). Male calling songs are composed of several series of single syllables with initial series having 2 to 4 syllables leading up to a sustained final series with about 25 syllables (Figs 2, 7). During the sustained portion of the final series the syllable rates are about ~5.8s⁻¹ at 25°C. Syllable rates change with temperature following the linear relationship: rate=0.293(temp)-1.467 (Fig. 8).

Amblycorypha peedee Forrest, sp. nov. https://zoobank.org/40A9EF19-9D67-481C-8EB3-6BBA1F0EFA99 Figs 5, 7–15, 17, Table 2

Material examined.—Holotype: USA ● ♂; North Carolina, Richmond Co., Sandhills Gamelands; 35.0528°N, 79.6035°W; 1 Jul. 2006; T.G. Forrest leg.; Ambar?-M03-2006; DNA (MS-044) NCBI accession SAMN31929325; REC (2006 Tape01 PGM 04); UNCA to be transferred to FSCA.

Allotype: USA • \bigcirc ; North Carolina, Richmond Co., Sandhills Gamelands; 35.06139°N, 79.63982°W; 16 Jul. 2004; T.G. Forrest leg.; Ambar?-F01-2004; **DNA** (NA); **REC** (NA); UNCA to be transferred to FSCA.

Paratypes: $(8 \circ, 0 \circ)$ USA • $1 \circ;$ North Carolina, Richmond Co.; 16 Jul. 2004; T.G. Forrest leg.; UNCA to be transferred to FSCA • $6 \circ;$ North Carolina: Richmond Co.; 1 Jul. 2006; T.G. Forrest leg.; UNCA to be transferred to FSCA • $1 \circ;$ North Carolina, Richmond Co.; 19 Jul. 2007; T.G. Forrest leg.; UNCA to be transferred to FSCA.

Other specimens: -23, 02: North Carolina specimens from Walker et al. 2003 deposited in Florida State Collection of Arthropods. USA • 13; North Carolina, Moore Co.; T.J. Walker leg.; (doublet song); FSCA • 13; North Carolina, Hoke Co; 26 Jul. 1964; T.J. Walker leg.; FSCA.

Size measurements (mm).—Holotype: PrnL: 5.9, PrnW: 4.45, TegL: 27.8, TegW: 10.0, HwEx: 3.9, FemL: 27.2, TibL: 28.8mm (Fig. 17). Allotype: PrnL: 7.0, PrnW: 46, TegL: 28.0, TegW: 9.6, HwEx: 2.2, FemL: 29.9, TibL: 31.0, OviL: 11.0mm.

Etymology.—This species is named for its geographic location, with populations north of the Pee Dee River, which isolates it from populations of *A. carolina*.

Common name.—Pee Dee round-winged katydid.

Differential diagnosis.—Amblycorypha peedee is best distinguished from other species in the rotundifolia complex by calling song. Syllables are almost always produced in doublets with syllable rates within doublets of about $11.6s^{-1}$ at $25 \,^{\circ}$ C (Fig. 8) and rates of $4.0s^{-1}$ at $25 \,^{\circ}$ C between doublets. The sustained portion of calling songs of *A. peedee* has about 17 syllables with 2–3 syllables in each of the series preceding the sustained portion (Fig. 7).



Fig. 17. Holotype of *Amblycorypha peedee*, PeeDee round-winged katydid. A. Dorsal view; B. Lateral view. Scale bars: 5 mm.

Description.—Individuals are typically green with characteristics of the *rotundifolia* complex of *Amblycorypha* (Walker et al. 2003). The male's calling song consists of a single syllable type that are produced in several (4–12) bouts of a 2–3 syllables preceding a sustained series of about 17 syllables (Figs 5, 7) that occur in pairs (doublets) (Fig. 5D). During the sustained portion of the song, at 25 °C the syllable rate between doublets is about 4.0s⁻¹ and is about 11.6s⁻¹ within the doublet. The function of syllable rate within a doublet in response to changes in temperature is rate=0.583(temp)-2.92 (Fig. 8). Female size measurements for the single individual that was collected are PrnL: 7.05; PrnW: 4.58; TegL: 28.0; TegW: 9.63; FemL: 29.9; TibL: 31.0; OviL: 11.0. Males' measurements (\bar{x} ±SE in mm, N) are on average PrnL: 5.84±0.04, 9; PrnW: 4.32±0.06, 9; TegL: 28.2±0.21, 9; TegW: 9.32±0.18, 9; FemL: 27.3±0.38, 6; TibL: 28.7±0.44, 6 (Table 2).

Amblycorypha tallapoosa Forrest, sp. nov.

https://zoobank.org/9F0FEE14-A7D9-45B6-8B72-CD2C52562766 Figs 3, 4, 7–15, 18, Table 2

Material examined.—Holotype: USA ● ♂; Alabama, Cleburne Co., Heflin, Talladega Nat Forest, CR 548; 33.78012°N, 85.52666°W; 2 Jun. 2007; T.G. Forrest leg.; Ambar-M05j-2007; DNA (MS-030) NCBI accession SAMN31929312; REC (2007 Tape03 PGM 10); UNCA to be transferred to FSCA.

Allotype: USA • \bigcirc ; Alabama, Cleburne, Pinhoti Trl, Coleman Lake; 33.78624 °N, 85.56705 °W; 2 Jun. 2007; T.G. Forrest leg.; Ambar-F02j-2007; DNA (MS-144) NCBI accession SAMN31929311; REC (2007 Tape03 PGM 05 duet with AmuGA-M01j-2007), REC (2007 Tape03 PGM 07 duet with Ambar-M04j-2007), REC (2007 Tape03 PGM 10 duet with Ambar-M05j-2007); UNCA to be transferred to FSCA.

Paratypes: $(6^{\circ}, 4^{\circ})$ USA • 1° ; Alabama, Cleburne Co.; 2 Jun. 2007; T.G. Forrest leg.; UNCA to be transferred to FSCA • 1°_{\circ} Alabama, Cleburne Co.; 3 Jun. 2007; T.G. Forrest leg.; UNCA to be transferred to FSCA • 2°_{\circ} ; Georgia, Gordon Co.; 9 Jul. 2005; J.A. Hamel and T. Richardson leg.; UNCA to be transferred to FSCA



Fig. 18. Holotype of *Amblycorypha tallapoosa*, Tallapoosa roundwinged katydid. A. Dorsal view; B. Lateral view. Scale bars: 5 mm.

• 2 $\[mathcal{P}, 1\]$; Georgia, Gordon Co.; 5 Jul. 2006; T.G. Forrest leg.; UNCA to be transferred to FSCA • 2 $\[mathcal{P}, 1\]$; Georgia, Gordon Co.; 1 Jun. 2007; T.G. Forrest leg.; UNCA to be transferred to FSCA.

Other specimens:—One specimen from Walker et al. 2003 •1♂; Alabama, Cleburne Co.; 29 Aug.1964, T.J. Walker leg.; FSCA.

Size measurements (mm).—Holotype: PrnL: 4.9, PrnW: 3.4, TegL: 24.3, TegW: 7.7, HwEx: 3.3, FemL: 23.7, TibL: 25.5mm (Fig. 18). Allotype: PrnL: 5.3, PrnW: 3.7, TegL: 24.1, TegW: 7.3, HwEx: 2.2, FemL: 26.5, TibL: 26.2, OviL: 9.2mm.

Common name.-Tallapoosa round-winged katydid

Etymology.—This species is named for its geographic location, with populations north of the Tallapoosa River and within the boundaries formed by its confluence with the Coosa River.

Differential diagnosis.—Although most of the size measurements of this species are smaller than the other eastern species we studied in this project (Table 2), calling songs are the best way to determine members of *A. tallaposa*. The syllable rates as a function of temperature for *A. tallaposa* males (~13s⁻¹ at 25°C) are the fastest among the species we studied (Fig. 8). Additionally, the main syllable series of the songs of *A. tallaposa* have fewer syllables (7.5±1) than the other species we studied (Fig. 7).

Description.—Individuals have all the characteristics typical of the *rotundifolia* complex (Walker et al. 2003). Size is generally small for the *rotundifolia* group. On average males' sizes are ($\bar{x}\pm$ SE in mm, N) PrnL: 5.08±0.14, 7; PrnW: 3.64±0.08, 7; TegL: 25.4±0.70, 7; TegW: 7.83±0.21, 7; FemL: 23.3±0.11, 7; TibL: 25.1±0.20, 7 and females are on average PrnL: 5.99±0.23, 5; PrnW: 3.86±0.13, 5; TegL: 26.0±0.67, 5; TegW: 8.07±0.34, 5; FemL: 25.9±0.49, 5; TibL: 26.6±0.59, 5; OviL: 10.7±0.38, 5 (Table 2). The main portion of the calling songs of males are series of about 8 syllables produced at rates of about 13s⁻¹ at 25°C (Figs 3, 4, 8). Preceding the sustained portion of the song,

males produce 1–6 shorter bouts of 3–8 syllables (Fig. 7). During the steady portion of the song, the relationship of syllable rate with changes in temperature (°C) is rate=0.750(temp)-5.86 (Fig. 8).

Future work.-More data from other geographic locations would help resolve several interesting questions. For example, why do populations of A. carolina differ so much genetically among the three sites we sampled in South Carolina? Also, it would be important to sample katydids on each side of the major rivers to determine the degree of isolation and reduction of gene flow. This would be particularly interesting around 1) the Pee Dee River where the doublet songs of A. peedee are found north of the river (Hoke Co., Richmond Co., Moore Co., NC) but not south of the river (Stanley Co., NC), 2) on either side of the Savannah River where song rates of A. carolina are much slower to the north (Edgefield Co., Aiken Co., and Georgetown Co., SC) than they presumably are to the south in GA, and 3) in AL where the calling songs of A. tallapoosa have fast rates in the region between the Coosa and Tallapoosa Rivers (Cleburne Co.) but have rates similar to FL A. bartrami farther south and west (Perry Co., AL see pink in Fig. 8).

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Supplementary material 1

Author: Timothy G. Forrest

Data type: xls

- Explanation note: Spreadsheet with morphological measurements of specimens that are included in the paper.
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Link: https://doi.org/10.3897/jor.32.96295.suppl1

Supplementary material 2

Author: Timothy G. Forrest

Data type: xls

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Author: Timothy G. Forrest

Data type: xls

- Explanation note: Spreadsheet containing counts of syllables for recordings in the paper.
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Supplementary material 4

Author: Timothy G. Forrest

Data type: xls

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A new record of a winged stick insect (Phasmatodea) from Mexico, with a checklist and key to the species of the family Pseudophasmatidae from continental North America

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Abstract

The winged stick insect *Metriophasma iphicles* (Redtenbacher, 1906) (Phasmatodea: Pseudophasmatidae) is recorded for the first time from Mexico (state of Veracruz), making this the northernmost record of both the species and genus. A checklist of species in the family Pseudophasmatidae from Mexico and the USA is presented, and a key to the species listed is proposed. With the current record, the number of continental North American species of Phasmatodea increases to 108, and the number of genera in the region increases to 23.

Keywords

Metriophasma iphicles, neotropics, phasmid, Phasmida, Veracruz

Introduction

The order Phasmatodea (stick and leaf insects) is currently comprised of more than 3500 species worldwide (Brock et al. 2022). In continental North America (Canada, USA, and Mexico), this order is represented by 107 species, grouped into 22 genera and 7 (potentially distantly related) families (López-Mora and Llorentes-Bousquets 2018, 2023, de Luna, in press). Among the North American phasmid fauna, there are records of only 5 winged species: 1 macropterous species of the genus Prisopus Peltier de Saint Fargeau & Audinet-Serville, 1827 (Prisopodidae: Prisopodinae: Prisopodini); 1 brachypterous species of the genus Haplopus Burmeister, 1838 (Phasmatidae: Cladomorphinae: Haplopodini); 2 brachypterous species of the genus Hypocyrtus Redtenbacher, 1908 (Phasmatidae: Cladomorphinae: Hesperophasmatini); and 1 macropterous species of the genus Agrostia Redtenbacher, 1906 (Pseudophasmatidae: Stratocleinae: Stratocleini). These winged species are mostly found in neotropical Mexico (Agrostia, Hypocyrthe United States, Florida (*Haplopus*) (Arment 2006, López-Mora and Llorente-Bousquets 2018).

The family Pseudophasmatidae is of the "Areolatae" group, meaning that its species possess an area apicalis; this is a sunken and usually triangular-shaped areola found in the ventral apex of the middle and hind tibiae (Bradley and Galil 1977, López-Mora and Llorente-Bousquets 2018). This character distinguishes the members of this family from most of the taxa of the region, except the members of the family Timematidae from which they differ most prominently in the number of tarsal segments: 5 in Pseudophasmatidae (and all other families) and 3 in Timematidae. Another exception is the members of the family Prisopodidae, from which they differ in the aspect of the last abdominal segments being laterally expanded into lobes in Prisopodidae (López-Mora and Llorente-Bousquets 2018). This family is currently divided into 3 subfamilies and 7 tribes: Pseudophasmatinae with 3 tribes, Anisomorphini, Paraprisopodini, and Pseudophasmatini; Stratocleinae with 1 tribe, Stratocleini; and Xerosomatinae with 3 tribes, Prexaspini, Setosini, and Xerosomatini (Brock et al. 2022). Pseudophasmatidae is represented in continental North America by 6 species: the macropterous Agrostia rugicollis (Gray, 1835); 3 apterous species of the genus Anisomorpha Gray, 1835 (Pseudophasmatinae: Anisomorphini), Anisomorpha buprestoides (Houttuyn, 1813), Anisomorpha ferruginea (Palisot de Beauvois, 1805), and Anisomorpha paromalus Westwood, 1859; and 2 apterous species of the genus Autolyca Stål, 1875 (Pseudophasmatinae: Anisomorphini), Autolyca elena Gorochov & Berezin, 2008 and Autolyca pallidicornis Stal, 1875. Agrostia rugicollis, Anisomorpha paromalus, and both species of Autolyca are found in neotropical Mexico; the remaining 2 species of Anisomorpha are found in southeastern USA (Arment 2006, López-Mora and Llorente-Bousquets 2018, de Luna in press). Until now, the genus Agrostia was the only recorded winged genus of this family in the region (Arment 2006, López-Mora and Llorente-Bousquets 2018).

species are mostly found in neotropical Mexico (*Agrostia, Hypocyrtus,* and *Prisopus*), but one is found in the southernmost state of Xerosomatinae: Prexaspini) contains 11 macropterous species

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that are distributed in the neotropical region. They are divided further into 2 subgenera: *Acanthometriotes* Hebard, 1924, which comprises 3 stocky species native of South America; and *Metrio phasma* Uvarov, 1940, which comprises 8 elongated species native mostly to South America. At least 2 species, *Metriophasma diocles* (Westwood, 1859) and *Metriophasma iphicles* (Redtenbacher, 1906), have been recorded in Central America (Brock et al. 2022). In the present publication, *Metriophasma iphicles* is recorded for the first time in Mexico, being found in the state of Veracruz; this is currently the northernmost distribution record of any species of the genus. A checklist of the species of the family Pseudophasmatidae from continental North America is presented, and a key to all listed species is proposed.

Methods

During a visit (July 2022) to the Estacion de Biologia Tropical "Los Tuxtlas", in the municipality of San Andres Tuxtla, state of Veracruz, Mexico, 8 specimens $(7 \overset{\circ}{\circ} \overset{\circ}{\circ}, 1 \overset{\circ}{\to})$ of a macropterous species of stick insect were collected at night (Fig. 1A). They were found perched on branches and vines, but there was no evidence indicating that the insects were feeding on these plants. The specimens were preserved individually in 70% ethanol and are kept at the Entomology Lab of the Facultad de Ciencias Forestales (FCF) of the Universidad Autonoma de Nuevo Leon (UANL) under the vouchers PHASM054–061, with 1 specimen being dry-mounted (PHASM056 – $\overset{\circ}{\circ}$ [Fig. 1B]) to obtain a better view of the pattern of the hindwings (Fig. 1B). Additionally, 3 other specimens from the same locality were examined; these are deposited in the Entomological Collection of the Estación de Biología Tropical "Los Tuxtlas" (EBTX45–47).

The keys of López-Mora and Llorente-Bousquets (2018) were employed in an attempt to identify the genus, the specimens keying to Perliodes (now a synonym of Agrostia). However, it was noted that the hindwings reached tergite IX (Figs 1B, 2A-C, 3A, C), while in Agrostia, it is known that the hindwings do not reach tergite VIII (Fig. 4A) (Aquino-Heleodoro et al. 2017). The presence of carinae in the middle and the hind femora was also noted, which are absent in all Stratocleinae including Agrostia (Zompro 2005). When the keys of Redtenbacher (1906) and Shelford (1909) were used instead, the specimens keyed to Metriotes (now a synonym of Metriophasma Uvarov, 1940), finding the same results when employing the key of Zompro (2005). After the genus was established, the keys to species present in the works of Redtenbacher (1906) and Shelford (1909) were employed, with the specimens keying to *Metriophasma iphicles*. Finally, the specimens were compared to photographs of the type material of Metriophasma iphicles from the Phasmida Species File website (Fig. 3A-C) (Brock et al. 2022), corroborating the identity of the collected material (Figs 1A, B, 2A-F).

Results and discussion

Checklist of species of Pseudophasmatidae from continental North America

The checklist includes records to state level, including those made or compiled by Redtenbacher (1906), Shelford (1909), Mariño and Marquez (1983), Conle and Hennemann (2002), Arment (2006), Gorochov and Berezin (2008), and López-Mora and Llorente-Bousquets (2018).



Fig. 1. *Metriophasma iphicles*, males. A. Live specimen found at night; wings spread; photo by Roberto García-Barrios; B. Dry-mounted specimen (PHASM056) with one tegmina and hindwing extended; photo by Manuel de Luna.

Family PSEUDOPHASMATIDAE Rehn, 1904 Subfamily Pseudophasmatinae Rehn, 1904 Tribe Anisomorphini Redtenbacher, 1906

Genus Anisomorpha Gray, 1835

1. *Anisomorpha buprestoides* (Houttuyn, 1813) USA (Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas).

2. Anisomorpha ferruginea (Palisot de Beauvois, 1805) USA (Delaware, Florida, Georgia, Illinois, Indiana, Kentucky, Louisiana, Mississippi, North Carolina, Nebraska [dubious record according to Arment 2006], Oklahoma, Pennsylvania, South Carolina, Texas, and Virginia).

3. Anisomorpha paromalus Westwood, 1859 MEXICO (Yucatan).

Genus Autolyca Stål, 1875

4. Autolyca elena Gorochov & Berezin, 2008 MEXICO (Chiapas).

5. Autolyca pallidicornis Stal, 1875 MEXICO (Chiapas).

Subfamily Stratocleinae Günther, 1953 Tribe Stratocleini Günther, 1953

Genus Agrostia Redtenbacher, 1906

6. Agrostia rugicollis (Gray, 1835) MEXICO (Colima).

Subfamily Xerosomatinae Bradley & Galil, 1977 Tribe Prexaspini Zompro, 2004

Genus Metriophasma Uvarov, 1940

7. *Metriophasma iphicles* (Redtenbacher, 1906) MEXICO (Veracruz). New record.

Material examined.-MEXICO • 1 2; Estación de Biología Tropical "Los Tuxtlas", municipality of San Andres Tuxtla, state of Veracruz; 18.5848°N, -95.0741°W, 147 m a.s.l.; 25 July 2022; on branches and vines; Roberto García-Barrios and Manuel de Luna leg.; wet specimen (70% ethanol); collected under permit SGPA/DGVS/04352/22; voucher PHASM054 (FCF-UANL) • 1 ♂; same data; voucher PHASM055 (FCF-UANL) ● 1 ♂; same data; dry-mounted specimen (Figs 2B, 3A–C); voucher PHASM056 (FCF-UANL) ● 1 ♂; same data; voucher PHASM057 (FCF-UANL) ● 1 ♂; same data; voucher PHASM058 (FCF-UANL) • 1 ♂; same data; voucher PHASM059 (FCF-UANL) ● 1 ♂; same data; voucher PHASM060 (FCF-UANL) • 1 3; same data; voucher PHASM061 (FCF-UANL) • 1 $\stackrel{\circ}{_+}$; same locality; 18.5831°N, -95.0741°W, 154 m a.s.l.; 24 September 2017; on Araceae; Ulises López Mora and Luis Rai Ruíz-Sánchez leg.; dry mounted; collected under permit SGPA/DGVS/03316/17; voucher EBTX45 (UNAM) • 1[♀]; same locality; 18.5847°N, -95.0735°W, 125 m a.s.l.; 25 September 2017; on Araceae; Ulises López Mora and Luis Rai Ruíz-Sánchez leg.; dry mounted; collected under permit SGPA/DGVS/03316/17; voucher EBTX46 (UNAM) ● ♂; same locality; 18.5862°N, -95.0768°W, 170 m a.s.l.; 18 August 2018; on Araceae; Ulises López Mora leg.; dry mounted; collected under permit SGPA/DGVS/002646/18; voucher EBTX47 (UNAM).



Fig. 2. *Metriophasma iphicles*, abdominal segments VIII, IX, and X. A. Dorsal aspect, male; B. Lateral aspect, male; C. Ventral aspect, male; D. Dorsal aspect, female; E. Lateral aspect, female; F. Ventral aspect, female. Photos by Manuel de Luna.



Fig. 3. *Metriophasma iphicles;* photographs by Dr. Paul D. Brock, copyright Natural History Museum of London. A. Dorsal aspect, male paralectotype; B. Lateral aspect, male paralectotype; C. Dorsal aspect, female lectotype.

New diagnosis.—*Metriophasma* has an area apicalis in the middle and hind tibiae, unlike all Diapheromeridae, *Parabacillus* Caudell, 1903, and Phasmatidae. It possesses 5-segmented tarsi, unlike the Timematidae. *Metriophasma* differs from all the North American genera, except 4 others, in having wings: the wings of *Metriophasma* are well-developed, unlike in the brachypterous species of *Haplopus* and *Hypocyrtus* (Phasmatidae). The abdomen of *Metriophasma* is not strongly lobed distally, as seen in macropterous species of *Prisopus* (Prisopodidae). Finally, *Metriophasma* has carinae on the

New diagnosis.—Metriophasma has an area apicalis in the middle ventral side of the middle and hind femora; these are lacking in all and hind tibiae, unlike all Diapheromeridae, *Parabacillus* Caudell, Stratocleinae, including *Agrostia* (Pseudophasmatidae) (Bradley 1903, and Phasmatidae. It possesses 5-segmented tarsi, unlike the and Galil 1977, Zompro 2005).

Metriophasma iphicles differs from the 3 species included in the subgenus Acanthometriotes for its elongated body and in having mesonotal carinae (Hebard 1924); from Metriophasma armatum (Redtenbacher, 1906), Metriophasma baculus (De Geer, 1773), and Metriophasma stollii (Gray, 1835) in having tubercles instead of spines in the dorsal aspect of the mesonotum (Redtenbacher



Fig. 4. Other North American representatives of the family Pseudophasmatidae; photographs by Dr. Paul D. Brock. A. *Agrostia rugicollis*, male syntype (type material of *Perliodes nigrogranulosus*, a synonym), copyright Naturhistorisches Museum Wien; B. *Autolyca pallidicornis*, male lectotype, copyright Naturhistorisches Museum Wien; C. *Anisomorpha paromalus*, male lectotype, copyright Natural History Museum of London.

1906); from *Metriophasma agathocles* (Stål, 1875), *Metriophasma baculus*, and *Metriophasma diocles* in having concolorous hindwings (Redtenbacher 1906, Shelford 1909); and from *Metriophasma pericles* (Redtenbacher, 1906) in not having a median carina in the mesonotum (Redtenbacher 1906).

This is the first time a species of the genus Metriophasma has been recorded for Mexico. The current record increases the number of species in continental North America to 108, the number of species of North American Pseudophasmatidae to 7, and the number of genera of the region to 23. Metriophasma iphicles had been previously recorded in the Central American countries of Honduras and Panama (Redtenbacher 1906, Shelford 1909), being the northernmost record of the species and genus. It is likely that this genus originated in South America, as this is where the majority of its species are found. At least 2 species reached Central America: Metiophasma diocles and Metriophasma iphicles. Following the tropical and subtropical areas found in the Atlantic versant, a population of Metriophasma iphicles reached Veracruz (Fig. 5). This distribution and tropical migration pathway has been observed in other animals, even those with low vagility, such as pit vipers (Saldarriága-Córdoba et al. 2017), so it is not surprising that a winged species could have easily followed it. The presence of this species is expected in more southern states, such as Chiapas or Oaxaca, as well as in northern Central American countries, such as Guatemala and Belize. Recently, there have been several instances of cryptic diversity in Phasmatodea; therefore, further molecular and morphological (internal genitalia) studies should follow to confirm whether this disjunct population is indeed Metriophasma iphicles or a closely related but undescribed species of the same species complex.



Fig. 5. Map of Mexico; pink circle points to the new record of *Metriophasma iphicles*.

Key to the North American Pseudophasmatidae

The following key works in adults of any sex from either the USA or Mexico. It follows keys and descriptions present in the works of Redtenbacher (1906), Shelford (1909), Conle and Hennemann (2002), Zompro (2005), Gorochov and Berezin (2008), and López-Mora and Llorente-Bousquets (2018). Care must be taken when examining specimens from neotropical Mexico, as some taxa might still be unreported or undescribed for this region.

- 1 Macropterous (tegmina reduced, hindwings well-developed, capable of flight) [Figs 1A, B, 3A–C, 4A]......2

- Hindwings shorter, not reaching the eighth abdominal tergite [Fig. 4A]; middle and hind femora without ventral carina. In the region, it has only been recorded in southwestern Mexico (Colima) Agrostia rugicollis

- 5 Pronotum slightly wider than long; ♂♂ mesonotum 1.8 times larger than wide, at max; ♀♀ mesonotum 1.4 times larger than wide, at max. Found in the Mexican Yucatan Penninsula.......*Anisomorpha paromalus*

Remarks on *Autolyca*: Some authors have stated that "the known representatives of *Autolyca* are invariably apterous" (Conle et al. 2009), which holds true for the majority of the described species. However, *Autolyca albifrons* Redtenbacher, 1906 was described to possess brachypterous hindwings but not tegmina (Redtenbacher 1906). The origin of the lone male holotype is unknown but is unlikely to be from New Caledonia (Redtenbacher even marked this dubious locality with "(?)"). Much more recently, Bank and Bradler (2022) mentioned an undescribed brachypterous species of *Autolyca* albifrons, the collection of more material, and the description of the undescribed Panamanian *Autolyca* are needed to begin resolving this matter.

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Mantodea of Iran: A review-based study

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Abstract

Scattered taxonomic data can be used to determine the geographic distribution of arthropods such as Mantodea (mantids). The distribution of mantids is not well known in Iran and not readily determined because the literature has been published in a mix of Persian-language and non-Persianlanguage scientific references, including books, journals, annual congress proceedings, and final reports of academic projects. To create a national checklist of mantids in Iran, I reviewed 35 Persian and non-Persian (English, German, and Italian) publications. I recorded 57 praying mantid species from 9 families described from localities across Iran. I identified 18 species—Ameles decolor, Ameles heldreichi, Ameles picteti, Ameles spallanzania, Elaea marchali, Empusa pennata, Eremiaphila andresi, Eremiaphila cerisy, Eremiaphila turcica, Geomantis larvoides, Iris coeca, Iris pitcheri, Oxyothespis wagneri, Pareuthyphlebs palmonii, Pseudoyersinia paui, Rivetina baetica, Severinia nigrofasciata, and Severinia turcomaniae-with records in Iran that may be incorrect based on geographic ranges that do not include Iran and similarity to other species that do occur in Iran. In the proposed checklist comprising 39 species, the family Rivetinidae, with 9 species, and the 2 families Amorphoscelidae and Nanomantidae, with 1 species each, comprised the greatest and least diversity, respectively. This checklist can facilitate future studies on Iran's mantodeans.

Keywords

checklist, Middle East, Persia, praying mantis

Introduction

Ancient records indicate that mantids have long been a part of human culture (Evans 2004), including humans in the Persian Plateau of Iran where mantids are depicted in ancient rock art (Kolnegari et al. 2020) and used in traditional medicine (Kolnegari pers. obs.). At 1,648,195 km², Iran is currently the 18th largest country and is located in the Middle East region of southwestern Asia. Iran is bordered to the north by Armenia, Azerbaijan, Turkmenistan, and the Caspian Sea; to the east by Afghanistan and Pakistan; to the south by the Gulf of Oman and the Persian Gulf; and to the west by Iraq and Turkey. About one-third of its 7,680km boundary is seacoast (Davoudzadeh 1997).

Iran includes three climatic zones: Mediterranean to the south, arid West Asian to the east and west, and temperate humid/semi-

humid Caspian zone to the north (Esmaeili et al. 2017). Several major biogeographical regions meet in this country, including the Palearctic, Eremic, and Oriental, which support a broad range of arthropod diversity (Zohary 1973, Olson et al. 2001). This includes a diversity of praying mantids that has been largely overlooked by native entomologists focused on species more important to agriculture (Kolnegari 2022).

Recently, mantid research in Iran has led to significant findings, including the identification of a new species (i.e., *Holaptilon brevipugilis* Kolnegari, 2018). This discovery highlights the potential for new discoveries resulting from more thorough taxonomic surveys in Iran. In anticipation of future taxonomic surveys, I undertook a literature review of Mantodea in Iran. Herein, I report the results, and in so doing, I provide the first checklist of the mantids of Iran.

Material and method

I reviewed the database of a national organization, the Iranian Research Institute of Plant Protection (IRIPP), which is regarded as the most important scientific organization working on arthropods in the country. The IRIPP's database consists of books, research articles, and annual congresses proceedings archived since 1946 (IRIPP 2022). I also reviewed two comprehensive books entitled Mantodea - Gottesanbeterinnen der Welt (Ehrmann 2002) and Praying Mantids; From Cognition to Conservation (Kolnegari 2022). Moreover, I used Google to search for the keywords "Mantodea", "mantis", "Iran", and "Persia" in English and Persian. I used these sources to create a national checklist of mantids in Iran. I adjusted the list to reflect current nomenclature and classification as indicated in the Mantodea Species File (Schwarz and Roy 2019, Otte et al. 2023) and in the latest relevant publications (Shcherbakov and Savitsky 2015, Villani 2020). In implementing these adjustments, I consolidated temporal and cross-language synonyms. I also documented records describing species that, if present in Iran, would be geographically highly isolated from their recognized ranges, and I identified these records as doubtful.

The global distribution of each species was assembled by consolidating the local or regional range maps provided by previous researchers (Kaltenbach 1963, Kaltenbach 1982, Ehrmann

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2002, Abu-Dannoun and Katbeh-Bader 2007, Ehrmann 2011, Kamal Mohammad et al. 2011, Caesar et al. 2015, Shcherbakov and Savitsky 2015, Akhmedov and Kholmatov 2019, Ali Panhwar et al. 2020, Villani 2020).

Results

I identified 57 species from nine families documented in 35 sources (Table 1). For around 80% of species, source material indicated a type location in a specific area, city, or province (n = 46)(Fig. 1). I consider 18 reported species-Ameles decolor (Charpentier, 1825), Ameles heldreichi Brunner von Wattenwyl, 1882, Ameles picteti (Saussure, 1869), Ameles spallanzania (Rossi, 1792), Elaea marchali (Reiche & Fairmaire, 1847), Empusa pennata (Thunberg, 1815), Eremiaphila andresi Werner, 1910, Eremiaphila cerisy Lefebvre, 1835, Eremiaphila turcica Westwood, 1889, Geomantis larvoides larvoides Pantel, 1896, Iris coeca Uvarov, 1931, Iris pitcheri Kaltenbach, 1982, Oxyothespis wagneri (Kittary, 1849), Pareuthyphlebs palmonii (Uvarov, 1939), Pseudoyersinia paui (Bolivar, 1898), Rivetina baetica baetica (Rambur, 1838), Severinia nigrofasciata Kaltenbach, 1982, and Severinia turcomaniae Saussure, 1872-as doubtful due to large separations between their recognized ranges, the locations indicated in Iran, and to their similarity to very similar species known to occur in Iran. Thus, the proposed checklist consists of 39 species with a high degree of certainty. The families Rivetinidae and Eremiaphilidae accounted for the highest number of Mantodeans in Iran (n = 9 and n = 8, respectively) (Table 2).

 Table 1. Literature used in the review-based study of Iran's Mantodea.

No. /	Scientific publication	No. /	Scientific publication
Code		Code	
1	Bagheri and Tajvand 2008	19	Mirzaee and Sadeghi 2021
2	Beier 1956	20	Mofidi-Neyestanak 2000
3	Bolivar 1911	21	Mofidi-Neyestanak 2015a
4	Bolivar 1913	22	Mofidi-Neyestanak 2015b
5	Brunner Von Wattenwyl 1878	23	Moradzadeh et al. 2021
6	Burr 1899	24	Morshedi Aghbolagh et al. 2012
7	Deeleman-Reinhold 1957	25	Rabieh et al. 2016
8	Ebner 1963	26	Ramme 1951
9	Ehrmann 2002	27	Rouhani et al. 2015
10	Ghahari and El-Den Nasser 2014	28	Sadeghi and Sadeghi 2015
11	Jamali and Mofidi-Neyestanak 2013	29	Sakenin et al. 2011
12	Kolnegari 2022	30	Samin et al. 2016
13	Kolnegari et al. 2022	31	Saussure 1870
14	Kolnegari and Vafaei-shoushtari 2018	32	Uvarov 1922
15	La Greca and Lombardo 1982	33	Uvarov 1938
16	La Greca and Lombardo 1987	34	Werner 1905
17	Mirzaee and Pashaei Rad 2017	35	Werner 1930
18	Mirzaee and Sadeghi 2019		

Table 2. Iran's Mantodea families and number of their species in Checklist of mantids of Iran the country.

Family	Number of species
Rivetinidae	9
Eremiaphilidae	8
Mantidae	6
Empusidae	4
Gonypetidae	4
Amelidae	3
Toxoderidae	3
Nanomantidae	1
Amorphoscelidae	1



Fig. 1. Map of Iran with administrative divisions (provinces) labeled and colored to indicate the relative diversity of Mantodea species (number of identified species). Abbreviations: Alborz (AL), Ardabil (AR), Bushehr (BU), Chaharmahal and Bakhtiari (CB), East Azerbaijan (EA), Fars (FA), Golestan (GO), Hamedan (HA), Hormozgan (HO), Ilam (IL), Iranian islands of Persian Gulf (IP), Isfahan (IS), Kerman (KE), Kermanshah (KS), Khuzestan (KH), Kohgiluyeh and Boyer-Ahmad (KB), Kurdistan (KU), Lorestan (LO), Markazi (MA), Mazandaran (MZ), North Khorasan (NK), Qazvin (QA), Qom (QO), Razavi Khorasan (RK), Semnan (SE), Sistan & Baluchistan (SB), South Khorasan (SK), Tehran (TE), West Azerbaijan (WA), Yazd (YA), Zanjan (ZA).

Institutional abbreviations.—

- BMNH British Museum, Natural History, London;
- HMIM Hayk Mirzayans Insect Museum, Tehran;
- IAUA Islamic Azad University of Arak, Markazi;
- INER Istituto Nazionale di Entomologia, Rome;
- Museo del Dipartimento di Biologia Animale dell'Università MBAC Catania;
- MHNG Muséum d'Histoire Naturelle, Geneva;
- MNMS Museo Nacional de Ciencias Naturales, Madrid;
- NHMW Naturhistorisches Museum, Vienna;
- NHRS Naturhistoriska Riksmuseet, Stockholm;
- NHTM Natural History and Technology Museum, Shiraz University;
- RMNH Nationaal Natuurhistorisch Museum, Leiden;
- Staatliches Museum für Naturkunde, Stuttgart; SMNS
- ZMSU Zoological Museum of Shiraz University, Fars.

The checklist is presented following alphabetic order. The list of references referring to a species are presented under backets after the species name.

Genus Aethalochroa Wood-Mason, 1877

1- Aethalochroa ashmoliana (Westwood, 1841) [2] [9] [21]

Other names.—Vates ashmoliana Westwood, 1841; Popa ashmoliana (Westwood, 1841); Arsacia ashmoliana (Westwood, 1841).

Iranian localities.—Sistan & Baluchistan Province (Iranshahr) [2].

Iranian repository.—HMIM [21].

Global distribution.-India, Iran, Pakistan, Sri Lanka, West Bengal.

Genus Ameles Burmeister, 1838

2- Ameles arabica Uvarov, 1939 [10]

Iranian localities.—Hormozgan Province (Haji-Abad) [10].

Iranian repository.—?

Global distribution.—Iran, Saudi Arabia.

3- Ameles persa Bolivar, 1911 [2] [3] [9] [10] [12] [17] [18] [19] [21] [25] [27] [28] Fig. 2G

Type locality.—Khuzestan Province (Kuh-sefid, Shimbar) [3].

Type specimen.—MNMS [3].

Iranian localities.—Fars Province [19] [28]; Fars Province (Shiraz) [12]; Isfahan Province (Ardestan) [10]; Kurdistan Province [27]; Kurdistan Province (Sanandaj) [12]; Lorestan Province (Kuhdasht) [18]; Markazi Province (Arak, Saveh, Shazand) [12]; Sistan & Baluchistan Province (Kuhe-Taftan, Makran, Sangan) [2]; South Khorasan Province [25]; Tehran Province [17].

Iranian repository.—HMIM [21]; IAUA [12]; NHTM [28]; ZMSU [18].

Global distribution.—Afghanistan, Armenia, Iran, Turkmenistan.

4- Ameles syriensis Giglio-Tos, 1915 [29] [30]

Iranian localities.—Ardabil Province (Meshkin-Shahr) [29]; Ilam Province (Ilam) [30].

Iranian repository.—?

Global distribution.—Iran, Jordan, Syria, Turkey.

Amorphoscelis Stål, 1871

5- Amorphoscelis pantherina Roy, 1966 [10] [12] [19] Fig. 2F

Iranian localities.—Hormozgan Province (Minab) [10]; Fars Province [12] [19].

Iranian repository.—?

Global distribution.—Iran, Iraq, Turkey.

Armene Stål, 1877

6- Armene pusilla (Eversmann, 1859) [10] [21]

Other names.—Mantis pusilla Eversmann, 1859.

Iranian localities.—Kurdistan Province (Bijar) [10].

Iranian repository.—HMIM [21].

Global distribution.—Afghanistan, Iran, Mongolia, Russia, Tajikistan, Turkmenistan, Uzbekistan.

Blepharopsis Rehn, 1902

7- Blepharopsis mendica (Fabricius, 1775) [2] [9] [10] [12] [18] [19] [21] [25] [29] Fig. 3C

Other names.—Mantis mendica Fabricius, 1775; Gryllus monstrosus Forskål, 1775; Blepharis mendica (Fabricius, 1775); Mantis dilaticollis Gistel, 1856.

Iranian localities.—Fars Province [19]; Fars Province (Khonj) [12]; Isfahan Province (Aran & Bidgol) [12]; Lorestan Province (Kuhdasht) [18]; Markazi Province (Saveh) [12]; North Khorasan Province (Bojnurd) [29]; Qom Province (Qom) [12]; Sistan & Baluchistan Province (Bampur [10], Konarak [12], Iranshahr, Khash, Kuhe-Taftan, Makran, Sangan [2]); South Khorasan Province [25]; Tehran Province [12]; Yazd Province (Naein) [12].

Iranian repository.—HMIM [21]; ZMSU [18].

Global distribution.—Afghanistan, Algeria, Canary Islands, Chad, Cyprus, Egypt, Ethiopia, NW India, Iran, Israel, Jordan, Lebanon, Libya, Mauritania, Morocco, Niger, Oman, Pakistan, Somalia, Sudan, Tunisia, Turkey, United Arab Emirates, Uzbekistan, Yemen.

Bolivaria Stål, 1877

8- Bolivaria brachyptera (Pallas, 1773) [2] [6] [9] [10] [12] [17] [18] [19] [21] [23] [25] [27] [28] [29] Fig. 2H

Other names.—Mantis brachyptera Pallas, 1773; Iris brachyptera (Pallas, 1773).

Iranian localities.—Fars Province [19] [28]; Gilan Province (Lahijan) [23]; Hamedan Province (Nahavand) [28]; Isfahan Province (Kashan [2], Khomeini Shahr [12]); Khuzestan Province (Izeh) [10]; Kurdistan Province [27]; Lorestan Province (Kuhdasht) [18]; Markazi Province (Arak, Shazand) [12]; Mazandaran Province [2]; South Khorasan Province [25]; Tehran Province [17]; Tehran Province (Ab-ali [2], Darakeh [12]); West Azerbaijan Province (Piranshahr [29], Kaboodan Island of Urmia Lake [6]).

Iranian repository.—HMIM [21]; IAUA [12]; NHTM [28]; ZMSU [18].

Global distribution.—Afghanistan, Armenia, Crete Island, Iran, Mongolia, Palestine, Russia, Turkey, Uzbekistan.

Elaea Stål, 1877

9- Elaea richteri Beier, 1956 [2] [8] [12] Fig. 2B

Type locality.—Sistan & Baluchistan Province (Iranshahr) [2].





Fig. 2. Photographs of some Mantodea species identified in Iran. A. *Empusa pennicornis;* B. *Elaea richteri;* C. *Iris oratoria;* D. *Eremiaphila persica;* E. *Oxyothespis persica;* F. *Amorphoscelis pantherina;* G. *Ameles persa;* H. *Bolivaria brachyptera;* I. *Iris nana.* Reprinted from M. Kolnegari "Praying Mantids.—From Cognition to Conservation" (Avaye Dornaye Khakestari Institute 2022).


Fig. 3. Photographs of some Mantodea species identified in Iran. A. Mantis religiosa; B. Empusa pennicornis; C. Blepharopsis mendica; D. Nilomantis floweri; E. Holaptilon brevipugilis. Reprinted from M. Kolnegari "Praying Mantids.—From Cognition to Conservation" (Avaye Dornaye Khakestari Institute 2022).

Type specimen.—SMNS [2].

Iranian localities.—Sistan & Baluchistan Province (Khash [8], Konarak [12]).

Iranian repository.—IAUA [12].

Global distribution.—Iran.

Empusa Illiger, 1798

10- Empusa fasciata Brullé, 1832 [9] [17] [19] [21] [23] [25] [27] [29] [30]

Iranian localities.—Fars Province [19]; Gilan Province (Lahijan) [23]; Ilam Province (Darrehshahr) [30]; Kermanshah Prov-

ince (Sahneh) [30]; Kurdistan Province [27]; Razavi Khorasan Province (Sarakhs) [29]; South Khorasan Province [25]; Tehran Province [17].

Iranian repository.—HMIM [21].

Global distribution.—Croatia, Cyprus, Egypt, Greece, India, Iran, Israel, Jordan, Romania, Russia, Slovenia, Turkey.

11- Empusa hedenborgii Stål, 1877 [2] [9] [10] [12] [17] [18]

Other names.—Empusa stollii Saussure, 1871.

Iranian localities.—Khuzestan Province (Ahwaz) [10]; Lorestan Province (Kuhdasht) [18]; Sistan & Baluchistan Province (Iranshahr [2], Konarak [12]); Tehran Province [17].

Iranian repository.—IAUA [12]; ZMSU [18].

Global distribution.—Cameroon, Egypt, Eritrea, Ethiopia, Iran, North Somalia, Saudi Arabia, Senegal, Sudan, United Arab Emirates, Yemen.

12- Empusa pennicornis Lindt, 1978 [2] [9] [10] [12] [17] [19] [20] [21] [22] [28] [29] [30] Figs 2A, 3B

Other names.—Mantis pennicornis Pallas, 1773; Gongylus marginatus Thunberg, 1815; Empusa orientalis Burmeister, 1838.

Iranian localities.—Ardabil Province (Meshkin-Shahr) [29]; Fars Province [19] [28]; Ilam Province (Mehran) [30]; Iranian islands of Persian Gulf [20]; Kermanshah Province (Javanrud) [30]; Kurdistan Province (Sanandaj) [10]; Markazi Province (Arak, Farahan, Khondab, Shazand) [12]; Sistan & Baluchistan Province (Khash, Kuhe-Taftan, Saravan) [2]; South Khorasan Province [2]; Tehran Province [17]; Tehran Province (Taleghan) [12].

Iranian repository.—HMIM [20] [21] [22]; IAUA [12]; NHTM [28].

Global distribution.—Afghanistan, China, Georgia, Iran, Iraq, Kazakhstan, Russia, Tajikistan, Turkey, Turkmenistan, Ukraine, Uzbekistan.

Eremiaphila Lefebvre, 1835

13- Eremiaphila arabica Saussure, 1871 [21]

Other names.—Eremiaphila dawydowi Werner, 1905.

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Egypt, Iran, Israel, Pakistan, Saudi Arabia, Yemen.

14- Eremiaphila gene Lefebvre, 1835 [9] [10] [25] [29]

Other names.—Eremiaphila burmeisteri Saussure, 1871; Eremiaphila hauensteini Werner, 1905.

Iranian localities.—East Azerbaijan Province (Arasbaran) [29]; Golestan Province (Gonbad) [29]; Isfahan Province (Isfahan) [10]; South Khorasan Province [25].

Iranian repository.—?

Global distribution.—Afghanistan, Armenia, Egypt, Iran, Israel, Jordan, Lebanon, Saudi Arabia, Syria, Turkey, Yemen.

15- Eremiaphila persica persica Werner, 1905 [2] [10] [12] [20] [21] [34] Fig. 2D

Type locality.—Khorasan Province [34].

Type specimen.—ZMAS [34].

Iranian localities.—Iranian islands of Persian Gulf [20]; Isfahan Province (Kashan) [2]; Kerman Province (Jiroft) [10]; Markazi Province (Arak) [12]; South Khorasan Province (Birjand) [2].

Iranian repository.—HMIM [20] [21]; IAUA [12].

Global distribution.—Azerbaijan, Iran, Iraq, Turkey.

- Eremiaphila persica sjostedti Werner, 1930 [21] [35]

Type locality.—Bushehr Province [35].

Type specimen.—NHRS [35].

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Iran, Pakistan.

Hierodula Burmeister, 1838

16- Hierodula macrostigmata Deeleman-Reinhold, 1957 [7]

Type locality.—Hormozgan Province (Jask) [7].

Type specimen.—RMNH [7].

Global distribution.—Iran.

17- Hierodula tenuidentata Saussure, 1869 [19] [29] [33]

Other names.—Hierodula simulacrum (Fabricius, 1793); Sphodromantis tenuidentata (Saussure, 1869); Hierodula heterodera Westwood, 1889.

Iranian localities.—Fars Province [19]; Tehran Province (Shahr-e Rey) [33].

Iranian repository.—?

Global distribution.—Afghanistan, India, Iran, Kazakhstan, Nepal, Pakistan, Tajikistan, Turkmenistan, Uzbekistan.

18- *Hierodula transcaucasica* Brunner von Wattenwyl, 1878 [2] [5] [10] [11] [12] [17] [21] [23] [24] [25] [29]

Iranian localities.—Ardabil Province (Dasht-e Moghan) [24]; Gilan Province [2]; Gilan Province (Lahijan) [23]; Kerman Province (Jiroft) [29]; Markazi Province (Arak) [12]; Mazandaran Province (Ghaemshahr, Juybar, Namak-abrood, Ramsar, Sari) [11]; Razavi Khorasan Province (Mashhad) [10]; Sistan & Baluchistan Province (Zabol) [10]; South Khorasan Province [25]; Tehran Province [2] [17].

Type locality.—Golestan Province (Gorgan) [5].

Type specimen.—NHMW [5].

Iranian repository.—HMIM [21] [24]; IAUA [12].

Global distribution.—Armenia, Azerbaijan, Caucasus, Georgia, Iran, Russia; invasive in southern Europe.

Holaptilon Beier, 1964

19- Holaptilon brevipugilis Kolnegari, 2018 [12] [14] Fig. 3E

Iranian localities.—Markazi Province (Arak) [12]; Markazi Province (Haftad-gholeh Protected Area) [14].

Iranian repository.—IAUA [12] [14].

Global distribution.—Iran.

Humbertiella Saussure, 1869

20- Humbertiella indica Saussure, 1869 [12]

Other names.—Humbertiella africana Rehn, 1912.

Iranian localities.—Sistan and Baluchistan Province (Konarak) [12].

Iranian repository.—IAUA [12].

Global distribution.—India, Iran, Myanmar, Nepal, Pakistan, Sri Lanka.

Iris Saussure, 1869

21- Iris nana Uvarov, 1930 [9] [12] [17] [19] [21] [33] Fig. 2I

Other names.-Iris radians Uvarov, 1931.

Iranian localities.—Bushehr (Delvar) [12]; Fars Province [19]; Fars Province (Marvdasht) [33]; Semnan Province (Semnan) [12]; Sistan & Baluchistan Province (Konarak) [12]; Tehran Province [17].

Iranian repository.—HMIM [21].

Global distribution.—Afghanistan, India, Iran, Iraq, Pakistan.

22- Iris oratoria (Linné, 1758) [9] [12] [17] [18] [19] [21] [24] [25] [26] [27] [28] [29] [30] Fig. 2C

Other names.—Mantis oratorius Linné, 1758; Mantis minima Charpentier, 1825.

Iranian localities.—Alborz Province (Shahrestanak) [12], Ardabil Province (Dasht-e Moghan) [24]; Fars Province [19] [28]; Kerman Province [26]; Kermanshah Province (Kermanshah) [30]; Kurdistan Province [27]; Kurdistan Province (Marivan) [12]; Lorestan Province (Kuhdasht) [18]; Markazi Province (Arak, Khondab, Shazand) [12]; North Khorasan Province (Bojnurd) [29]; South Khorasan Province [25]; Tehran Province [26] [17].

Iranian repository.—HMIM [21] [24]; IAUA [12]; NHTM [28]; ZMSU [18].

Global distribution.—Albania, Algeria, Chad, Croatia, Cyprus, Egypt, France, Greece, India, Iran, Israel, Italy, Jordan, Morocco, North America, Palestine, Spain, Syria, Tunisia, Turkey; invasive in southwestern USA.

23- Iris persa Uvarov, 1922 [2] [8] [21] [32]

Type locality.—Bushehr Province [32].

Type specimen.—BMNH [32].

Iranian localities.—Fars Province (Shiraz) [8]; Sistan & Baluchistan Province (Iranshahr, Khash, Kuhe-Taftan, Saravan) [2].

Iranian repository.—HMIM [21].

Global distribution.—Iran.

24- Iris polystictica (Fischer-Waldheim, 1846) [9] [11] [12] [17] [19] [21] [24]

Other names.—Mantis polystictica Fischer-Waldheim, 1846; Iris tiflisina Giglio-Tos, 1915.

Iranian localities.—Ardabil Province (Dasht-e Moghan) [24]; Fars Province [19]; Tehran Province [12] [17]; Mazandaran Province (Ghaem-shahr, Juybar, Sari) [11].

Iranian repository.—HMIM [11] [21] [24].

Global distribution.—Afghanistan, Armenia, Azerbaijan, Caucasus, China, Georgia, Iran, Kazakhstan, Russia, Tajikistan, Turkey, Turkmenistan, Uzbekistan.

25- Iris splendida Uvarov, 1923 [10] [21] [25]

Iranian localities.—Fars Province (Shiraz) [10]; Hormozgan Province (Bandar-Abbas) [10]; South Khorasan Province [25].

Iranian repository.—HMIM [21].

Global distribution.—Afghanistan, Iran, Pakistan.

Lobothespis La Greca & Lombardo, 1987

26- Lobothespis vignai La Greca & Lombardo, 1987 [16]

Type locality.—Sistan & Baluchistan Province (Hamun) [16].

Type specimen.—INER [16].

Global distribution.—Iran.

Mantis Linné, 1758

27- Mantis religiosa Linnaeus, 1758 [1] [2] [8] [9] [10] [11] [12] [13] [17] [18] [19] [20] [21] [24] [25] [27] [28] [29] [30] Fig. 3A

Other names.—Gryllus religiosus Linné, 1758; Mantis sancta Fabricius, 1787; Mantis maroccana Thunberg, 1815; Mantis capensis Saussure, 1872.

Iranian localities.—Ardabil Province (Dasht-e Moghan) [24]; East Azerbaijan Province (Arasbaran) [29]; Fars Province [19] [28]; Fars Province (Kazeroon, Shiraz) [8] [10]; Hormozgan Province

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(Bandar-Abbas) [10]; Ilam Province (Dehloran [10], Ilam [30]); Iranian islands of Persian Gulf [20]; Kerman Province (Manujan) [13]; Kermanshah Province (Javanrud, Kermanshah) [30]; Khuzestan Province [1]; Kurdistan Province [27]; Kurdistan Province (Bijar) [10]; Lorestan Province (Kuhdasht) [18]; Mazandaran Province [2] [12]; Mazandaran Province (Ghaem-shahr, Chalous, Juybar, Mahmood-abad, Namak-abrood, Sari, Savadkuh) [11]; Razavi Khorasan Province (Mashhad, Sabzevar) [10] [29]; South Khorasan Province [25]; Sistan & Baluchistan Province (Iranshahr) [2]; Tehran Province [12] [17]; West Azerbaijan Province (Urmia) [8] [29].

Iranian repository.—HMIM [11] [20] [21] [24]; NHTM [28]; ZMSU [18].

Global distribution.—Africa, Asia, Europe. Invasive in North America.

Microthespis Werner, 1908

28- Microthespis dmitriewi Werner, 1908 [2] [9] [18] [19] [21] [30]

Iranian localities.—Fars Province [19]; Kermanshah Province (Sahneh) [30]; Lorestan Province (Kuhdasht) [18]; Sistan & Baluchistan Province (Iranshahr, Saravan) [2].

Iranian repository.—HMIM [21]; ZMSU [18].

Global distribution.—Bahrain, Egypt, Ethiopia, Iran, Israel, Jordan, Oman, Pakistan, Saudi Arabia, Somalia, United Arab Emirates, Yemen.

29- Microthespis evansi Uvarov, 1931 [21]

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Iran, Pakistan.

Nilomantis Werner, 1907

30- *Nilomantis floweri* Werner, 1907 [9] [12] [21] Fig. 3D

Other names.—Cryptomantis tenella Giglio-Tos, 1915; Nilomantis arabica Beier, 1930.

Iranian localities.—Fars Province [12]; Hormozgan Provincen (Gheshm) [12].

Iranian repository.—HMIM [21].

Global distribution.—Chad, Ethiopia, Iran, Mauritania, Nigeria, Oman, Saudi Arabia, Sudan, United Arab Emirates, Yemen.

Oxyothespis Saussure, 1870

31- Oxyothespis persica Bolivar, 1913 [2] [4] [10] [12] [18] [19] [20] [21] [22] [25] Fig. 2E *Type specimen.*—MNMS [4].

Iranian localities.—Iranian islands of Persian Gulf [20]; Isfahan Province (Najaf-Abad) [10]; Fars Province [19]; Fars Province (Shiraz) [12]; Lorestan Province (Kuhdasht) [18]; Sistan & Baluchistan Province (Iranshahr, Makran) [2]; South Khorasan Province [25].

Iranian repository.—HMIM [20] [21] [22]; ZMSU [18].

Global distribution.—Iran, Pakistan.

Rivetina Berland & Chopard, 1922

32- Rivetina caucasica caucasica (Saussure, 1871) [9] [18] [29]

Other names.—Iris caucasica Saussure, 1871; Fischeria caucasica Saussure, 1871.

Iranian localities.—Lorestan Province (Kuhdasht) [18]; West Azerbaijan Province (Maku) [29].

Iranian repository.—ZMSU [18].

Global distribution.—Caucasus, Iran, Syria, Tajikistan, Turkey.

33- Rivetina dolichoptera (Schulthess-Rechberg, 1894) [2] [9]

Other names.—Bolivaria dolichoptera Schulthess-Rechberg, 1894; Fischeria dolichoptera (Schulthess-Rechberg, 1894).

Iranian localities.—Sistan & Baluchistan Province (Iranshahr, Khash, Kuhe-Taftan, Saravan) [2]; South Khorasan Province (Birjand) [2].

Iranian repository.—?

Global distribution.—Iran, Pakistan, Saudi Arabia, United Arab Emirates, Yemen.

34- Rivetina excellens Beier, 1956 [2]

Type locality.—Sistan & Baluchistan Province (Iranshahr) [2].

Type specimen.—SMNS [2].

Global distribution.—Iran, Iraq, United Arab Emirates, Yemen.

35- Rivetina inermis inermis (Uvarov, 1923) [19] [21]

Other names.—Fischeria inermis Uvarov, 1923.

Iranian localities.—Fars Province [19].

Iranian repository.-HMIM [21].

Global distribution.—Iran, Saudi Arabia, Yemen.

- Rivetina inermis iranica La Greca & Lombardo, 1982 [15]

Type locality.—Bushehr Province [15].

Type specimen.—MBAC [15].

Type locality.—Khuzestan Province (Gotvand, Shimbar) [4].

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Global distribution.—Bahrain, Iran.

- Rivetina inermis (not assigned to subspecies) [25] [28]

Iranian localities.—Fars Province [28]; South Khorasan Province [25].

Iranian repository.-NHTM [28].

36- Rivetina rhombicollis La Greca & Lombardo, 1982 [15]

Type locality.—Sistan and Baluchistan Province [15].

Type specimen.—MBAC [15], SMNS [15].

Global distribution.—Afghanistan, Iran, Pakistan.

37- Rivetina syriaca syriaca (Saussure, 1869) [21]

Other names.—Iris syriaca Saussure, 1869; Fischeria festae Giglio-Tos, 1916; Eufischeriella festae (Giglio-Tos, 1916); Rivetina festae (Giglio-Tos, 1916).

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Iran, Iraq, Lebanon, Syria, Tajikistan, Transcaspia, Turkey.

Sphodromantis Stål, 1871

38- Sphodromantis trimacula (Saussure, 1870) [31]

Other names.—Hierodula trimacula Saussure, 1870; Hierodula arabica Wood-Mason, 1882; Sphodromantis arabica Wood-Mason, 1882; Sphodromantis dhufarica Uvarov, 1933.

Type locality.—unknown.

Type specimen.—BMNH [31]; MHNG [31].

Global distribution.—Egypt, Iran, Iraq, Lebanon, Oman, Saudi Arabia, United Arab Emirates, Yemen.

39- Sphodromantis viridis (Forskål, 1775) [11] [12] [21] [24] [29] [30]

Other names.—Gryllus viridis Forskål, 1775; Mantis guttata Thunberg, 1815; Mantis bioculata Burmeister, 1838; Hierodula bioculata (Burmeister, 1838); Sphodromantis bioculata (Burmeister, 1838).

Iranian localities.—Ardabil Province (Dasht-e Moghan) [24]; East Azerbaijan Province (Arasbaran) [29]; Fars Province (Shiraz) [12]; Kermanshah Province (Kermanshah) [30]; Mazandaran Province (Ghaem-shahr, Kelardasht, Ramsar, Sari, Savadkuh, Shirgah) [11]; Sistan and Baluchistan Province (Konarak) [12].

Iranian repository.—HMIM [11] [21] [24].

Global distribution.—Algeria, Chad, Croatia, Cyprus, Egypt, Ethiopia, Jordan, Iran, Israel, Kenya, Libya, Mauretania, Morocco,

Palestine, Saudi Arabia, Senegal, Somalia, Spain, Sudan, Syria, Tanzania, Tunisia, Uganda, Yemen.

Species likely misidentified in primary literature

1- Ameles decolor (Charpentier, 1825) [21] [24]

Other names.—Mantis decolor Charpentier, 1825.

Iranian localities.-Ardabil Province (Dasht-e Moghan) [24].

Iranian repository.—HMIM [21] [24].

Global distribution.—Albania, Algeria, Croatia, Czech Republic, France, Greece, Italy, Slovenia, Spain.

Note.—The easternmost occurrence of the species is along the northeastern coast of the Adriatic Sea. No confirmed Asian records of this species exist (Kaltenbach 1963, Agabiti et al. 2010, Villani 2020).

2- Ameles heldreichi Brunner von Wattenwyl, 1882 [29]

Other names.—Parameles heldreichi (Brunner von Wattenwyl, 1882).

Iranian localities.—East Azerbaijan Province (Arasbaran) [29].

Iranian repository.—?

Global distribution.—Cyprus, Greece, Israel, Jordan, Libya, Palestine, Turkey.

Note.—The easternmost distribution of the species includes Crimea, Central Anatolia, and the eastern Mediterranean coast.

3- Ameles picteti (Saussure, 1869) [10] [21]

Other names.—Parameles picteti Saussure, 1869; Mantis nana Charpentier, 1825.

Iranian localities.—Semnan Province (Shahrud) [10].

Iranian repository.—HMIM [21].

Global distribution.—Algeria, Italy, Morocco, Spain.

Note.—This species has not been recorded away from western Mediterranean coasts (Agabiti et al. 2010).

4- Ameles spallanzania (Rossi, 1792) [21]

Other names.—Mantis spallanzania Rossi, 1792; *Mantis nana* Charpentier, 1825; *Ameles abjecta* Bolivar, 1897.

Iranian localities.—unknown.

Iranian repository.-HMIM [21].

Global distribution.—Albania, Algeria, Croatia, France, Greece, Italy, Libya, Morocco, Portugal, Spain, Tunisia.

Note.—The easternmost occurrence of this species is Cyprus (Agabiti et al. 2010).

5- Elaea marchali (Reiche & Fairmaire, 1847) [21]

Other names.—Eremiaphila marchali Reiche & Fairmaire, 1847; Humbertiella perloides Saussure, 1869; Elaea perloides (Saussure, 1869); Elaea somalica Schulthess-Schindler, 1898.

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Algeria, Cameroon, Egypt, Ethiopia, Guinea, Kenya, Nigeria, Senegal, Sudan.

Note.—This species is mainly found throughout the savanna regions of Northern Africa. No confirmed records of this species exist outside the Ethiopian region.

6- Empusa pennata (Thunberg, 1815) [2] [9] [10] [11] [21] [24]

Other names.—Gongylus pennatus Thunberg, 1815; Empusa pauperata (Fabricius, 1781); Empusa egena Charpentier, 1841; Empusa brachyptera Fischer-Waldheim, 1846.

Iranian localities.—Ardabil Province (Dasht-e Moghan) [24]; Mazandaran Province (Ghaem-shahr, Namak-abrood, Ramsar, Savad-kuh, Shirgah) [11]; Sistan & Baluchistan Province (Iranshahr [2], Zahedan [10]).

Iranian repository.—HMIM [21].

Global distribution.—Algeria, Italy, Libya, Morocco, Spain, Tunisia.

Note.—This is a western Mediterranean species. The easternmost distribution of it includes Istria (Kaltenbach 1963).

7- Eremiaphila andresi Werner, 1910 [30]

Iranian localities.-Ilam Province (Darrehshahr) [30].

Iranian repository.—?

Global distribution.-Egypt, Iraq, Libya.

8- Eremiaphila cerisy Lefebvre, 1835 [9]

Iranian localities.—unknown.

Iranian repository.—?

Global distribution.—Egypt, Iraq, Oman, Saudi Arabia, United Arab Emirates.

9- Eremiaphila turcica Westwood, 1889 [9]

Iranian localities.—unknown.

Iranian repository.—?

Global distribution.—Iraq, Turkey.

10- Geomantis larvoides larvoides Pantel, 1896 [29]

Other names.—Fischeria baetica Pantel, 1886.

Iranian localities.—Gilan Province (Astara) [29].

Iranian repository.—?

Global distribution.—Albania, Croatia, France, Greece, Italy, Morocco, North Africa, Portugal, Spain, Tunisia, Turkey.

Note.—This species has not been recorded away from Mediterranean coasts.

11- Iris coeca Uvarov, 1931 [10] [21]

Iranian localities.—Ilam Province (Dehloran) [10].

Iranian repository.—HMIM [21].

Global distribution.—Egypt, Saudi Arabia, Sudan, Yemen.

12- Iris pitcheri Kaltenbach, 1982 [21]

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Saudi Arabia.

13- Oxyothespis wagneri (Kittary, 1849) [9]

Other names.—Mantis wagneri Kittary, 1849.

Iranian localities.—unknown.

Iranian repository.—?

Global distribution.—Afghanistan, Kazakhstan.

14- Pareuthyphlebs palmonii (Uvarov, 1939) [21]

Other names.—Xenomantis palmonii Uvarov, 1939.

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Israel, Jordan, Palestine.

Note.—Distribution of the species is limited to distinct regions of the Middle East along Mediterranean coasts.

15- Pseudoyersinia paui (Bolivar, 1898) [24]

Other names.—Ameles paui Bolivar, 1898; Parameles paui (Villani 2020).

Iranian localities.—Ardabil Province (Dasht-e Moghan) [24].

Iranian repository.-HMIM [24].

Global distribution.—Spain.

Note.—This species has been recorded only in Spain.

16- Rivetina baetica baetica (Rambur, 1838) [12] [21] [22] [29]

Other names.—Mantis baetica Rambur, 1838; Mantis fasciata Thunberg, 1815; Fischeria baetica (Rambur, 1838).

Iranian localities.—East Azerbaijan Province (Arasbaran) [29]; Markazi Province (Arak, Farahan) [12].

Iranian repository.—HMIM [21] [22]; IAUA [12].

Global distribution.—Algeria, Chad, Egypt, Italy, Libya, Malta, Mauritania, Morocco, Senegal, Spain, Tunisia.

Note.—This species mainly occurs in Northern Africa and Southern Europe (La Greca and Lombardo 1982).

17- Severinia nigrofasciata Kaltenbach, 1982 [21]

Iranian localities.—unknown.

Iranian repository.—HMIM [21].

Global distribution.—Saudi Arabia.

18- Severinia turcomaniae Saussure, 1872 [28]

Other names.—Oxythespis turcomaniae Saussure, 1872.

Iranian localities.—Fars Province [28].

Iranian repository.—NHTM [28].

Global distribution.—Mongolia, Turkestan.

Discussion

This study demonstrates the potential significance of Iranian repositories for future investigations. However, to validate the primary identification of Iranian repositories, the specimens need to be properly preserved. Unfortunately, some have been poorly preserved in local institutions where they are prone to accidental destruction or loss (Kolnegari pers. obs.). The Hayk Mirzayans Insect Museum (HMIM) has solved these problems by limiting access to voucher specimens. This ensures conservation of the largest insect collection of Iran with over four million specimens (IRIPP 2022) but simultaneously creates a challenge to documenting the history and distribution of mantids in Iran. At least 35 mantid specimens likely housed in the HMIM should be included in future study of the Mantodea of Iran.

Although the number of mantids documented in Iran is relatively small to date (i.e., approximately 2% of globally identified mantids), the large size of the country and the relative paucity of information on its invertebrate community make it likely that Iran contains undocumented mantid taxa. This is particularly true of the western half of Iran where two main mountain ranges (Alborz and Zagros) have been described as cradles of unique floristic and faunistic diversity (Zohary 1973, Esmaeili et al. 2017). Systematic surveys appear to have never occurred in four

provinces in western Iran, including Chaharmahal and Bakhtiari, Kohgiluyeh and Boyer-Ahmad, Qazvin, and Zanjan (Fig. 1). Moreover, each of the 17 known ecoregions of Iran—particularly marginal ecoregions—could be highly important from a zoogeographical perspective (Olson et al. 2001), but they were not considered in previous studies. Therefore, conducting systematic surveys in mantid habitats in the unexplored provinces and in all ecoregions of Iran could lead to taxonomically and faunistically significant achievements.

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Geographic variation in phenotypic divergence between two hybridizing field cricket species

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Abstract

Patterns of morphological divergence across species' ranges can provide insight into local adaptation and speciation. In this study, we compared phenotypic divergence among 4,221 crickets from 337 populations of two closely related species of field cricket, Gryllus firmus and G. pennsylvanicus, and their hybrids. We found that these species differ across their geographic range in key morphological traits, such as body size and ovipositor length, and we directly compared phenotype with genotype for a subset of crickets to demonstrate nuclear genetic introgression, phenotypic intermediacy of hybrids, and essentially unidirectional mitochondrial introgression. We discuss how these morphological traits relate to life history differences between the two species. Our comparisons across geographic areas support prior research suggesting that cryptic variation within G. firmus may represent different species. Our study highlights how variable morphology can be across wide-ranging species and the importance of studying reproductive barriers in more than one or two transects of a hybrid zone.

Keywords

Gryllus, hybrid zone, local adaptation, morphology, Orthoptera, speciation

Introduction

Phenotypic divergence can provide insight into evolutionary processes acting across different scales of biological organization. Within a single species, phenotypic divergence can reflect differences between environments, population histories, or a combination of these factors (Gavrilets et al. 2001, Uyeda et al. 2009, Runemark et al. 2010, Oneal and Knowles 2013, Jenck et al. 2020). Phenotypic divergence can signal the possible early stages of species differentiation (Wolf et al. 2008, González et al. 2011, Skoglund et al. 2015) and, in closely related species, can shed light on local adaptation and patterns of increasing divergence (Britch and Cain 2001, Shaw and Mullen 2011). Most studies of species divergence have limited replication across the ranges of a species pair, and the specific traits that maintain reproductive barriers between species are not always clear (Harrison and Larson 2016). Geographically comprehensive surveys of phenotypic divergence are much harder (Jiménez and Ornelas 2015, Wang et al. 2017, Polly and Wójcik 2019, Moran et al. 2020) but critical if we are to understand the origin and maintenance of species' boundaries.

The relationship between divergent phenotypic characteristics and reproductive barriers is most easily studied in places where the ranges of closely related species overlap and heterospecific individuals mate and produce offspring (Barton and Hewitt 1985, Harrison 1990). In the resulting hybrid zone, as the different species co-exist, compete, and interbreed, phenotypic characteristics may be more variable among individuals when compared to the pure allopatric populations that lie outside the hybrid zone (Hollander et al. 2018, Sottas et al. 2018). By comparing the phenotypic variation between both conspecific allopatric and sympatric populations and between heterospecific populations, it becomes possible to examine the potential causes of phenotypic evolution, speciation, and how those mechanisms lead to the reproductive barriers that maintain species boundaries (Shaw and Mullen 2011).

In this study, we examined the phenotypic divergence between two closely related and geographically widespread species of North American field crickets, *Gryllus pennsylvanicus* Burmeister 1838 and *G. firmus* Scudder 1902, whose common ancestry dates to roughly 200,000 years ago (Willett et al. 1997, Maroja et al. 2009a). The more northern, inland species, *G. pennsylvanicus*, is

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broadly distributed throughout the United States, while the more southern, coastal species, G. firmus, is restricted to the east coast and west into Texas (Alexander 1968, Harrison and Arnold 1982, Weissman and Gray 2019). These species form a hybrid zone along the eastern front of the Appalachian Mountains (Harrison and Arnold 1982), and where they co-occur, they are isolated by multiple reproductive barriers. The most striking barrier is a oneway incompatibility: G. firmus females mated to G. pennsylvanicus males lay few eggs that do not hatch (Harrison 1983, Maroja et al. 2009b, Larson et al. 2012). These two species are also isolated by habitat: G. firmus is often found in sandy habitats and has lighter coloration and longer ovipositors that can presumably lay eggs deeper in sandy soils (Harrison 1986, Ross and Harrison 2006). Also, Gryllus firmus is a larger cricket, though size may vary with the length of the growing season (Masaki 1961). In some parts of the hybrid zone, G. firmus develops faster and emerges earlier in the season, leading to temporal isolation (Harrison 1985).

These morphological differences have been well characterized in a handful of locations within the hybrid zone (e.g., Connecticut), but whether these morphological traits are consistently different between G. firmus and G. pennsylvanicus remains an open question (Weissman and Gray 2019). When species differences are studied in only a few locations, it may be impossible to distinguish species-specific traits from within-species local adaptation. Morphological traits, such as lighter color and longer ovipositors, may have evolved in specific areas due to habitat selection. Likewise, body size may vary with climate and latitude. This paper presents the first geographically comprehensive comparison of G. firmus and G. pennsylvanicus by combining published and unpublished morphological datasets for these two species across their geographic ranges. Our dataset includes 4,221 crickets from 337 populations, spanning collections over four decades. We had three objectives. First, we quantified morphological divergence within and between species across their geographic ranges. Second, for populations near the hybrid zone, we tested whether traits that distinguish species correlate with ancestry. Finally, we examined

the correlation between morphological traits and environmental variables across the ranges of these species. In doing so, we aimed to gain a greater understanding of how population variation and local adaptation contribute to divergence and speciation.

Materials and methods

Cricket collections.—We compiled a dataset of 4,221 crickets, the majority being G. pennsylvanicus but also G. firmus and their hybrids, from 337 collecting localities (Fig. 1). Crickets were sampled throughout the United States and Canada, with the largest collections coming from the northeastern United States and the hybrid zone. Sampling spanned 40 years (1983-2022), with collections performed by A.R. Byerly, E.L. Larson, L.S. Maroja, C.L. Ross, and R.G. Harrison. In addition to these previously unpublished morphological data, we included data from Ross and Harrison (2002), Larson et al. (2013), and Weissman and Gray (2019), with the latter being the most geographically widespread dataset. We also included morphological data from a newly described cricket species, G. thinos Weissman and Gray 2019, , which is closely related to G. pennsylvanicus and G. firmus (Gray et al. 2020). We included G. thinos to enable us to compare morphological variation within G. firmus to that of a closely related species that occupies the same habitat but is classified as a separate species.

We categorized each collecting location as allopatric or sympatric based on past sampling of the field cricket hybrid zone (Harrison and Arnold 1982, Willett et al. 1997, Maroja et al. 2009a, Larson et al. 2013a, 2014). Populations in and near the hybrid zone often have individuals that are pure *G. firmus* or pure *G. pennsylvanicus*, but they also have many backcrosses and recent generation hybrids (Harrison and Bogdanowicz 1997, Maroja et al. 2009a, Larson et al. 2013a, 2014). Because of this, we considered any collecting locations that were near the hybrid zone to be "sympatric". We also assigned each collecting location to a geographic region (labeled in Fig. 1). These regions, identified using climatological data (Karl and Koss 1984), were as follows: central (**CTR**: IL, IN, KY, MO, OH, TN,



Fig. 1. Map of North American cricket collecting locations. Allopatric populations of *Gryllus firmus* are in yellow, *G. pennsylvanicus* are in teal, *G. thinos* populations are in purple, and sympatric *G. firmus* and *G. pennsylvanicus* populations are in red. The size of the circle corresponds to the sample size for each location. **A.** Entire range of collection locations in the United States and Canada; **B.** Enlarged area of densely sampled locations in northeast, central, and southeast United States.

WV); east north central (ENC: IA, MI, MN, WI); northeast (NE: CT, DE, ME, MD, MA, NH, NJ, NY, PA, RI, VT); northwest (NW: ID, OR, WA); south (SO: AR, KS, LA, MS, OK, TX); southeast (SE: AL, FL, GA, NC, SC, VA); southwest (SW: AZ, CO, NM, UT); west (WE: CA, NV); and west north central (WNC: MT, NE, ND, SD, WY).

In all cases, crickets were collected by hand and maintained in plastic containers with food (cat and rabbit food), water vials, and shelter prior to freezing. Most samples were collected as adults, but in some cases, crickets were collected as late instar nymphs. Nymphs were allowed to mature to the adult stage in the laboratory before freezing. Most collections were done in August–September, but some crickets were collected in late July or early October.

Morphological measurements.—We focused only on traits that were measured using the same methods across different studies. Crickets were measured for body size, as gauged by either body length, femur length, and/or pronotum width. Body length was measured from the vertical surface of the face to the tip of the abdomen, straightening the body when necessary. Pronotum width was measured at the widest part of the pronotum. Femur length was measured from the proximal to distal end of the hind femur. Female ovipositor length was measured from the point of attachment on the abdomen to the distal end of the ovipositor. Because ovipositor length varies isometrically with body size (Suppl. material 1: fig. S1), we also calculated relative ovipositor length as the length of the ovipositor divided by pronotum width or femur length, depending on sample availability. We obtained all measurements using Vernier calipers and recorded values to the nearest 0.1 mm.

For a subset of samples where tegmina were available (31 allopatric crickets and 437 sympatric crickets), we measured their color using a USB4000 spectrophotometer with an Ocean Optics PX-2 pulsed xenon lamp and SpectraSuite v2.0 software. We mounted a probe on a metal stand at a 90° angle 0.7 mm from the surface of the tegmina. For each male, we recorded and averaged spectral reflectance for three points near the center of the tegmina. We recorded spectral measurements as the percentage of reflected light relative to a Spectralon white standard, restricted our analyses to wavelengths of 300 700 nm, and used a segmental classification method to estimate brightness, chroma, and hue using CLR v1.1 (Montgomery 2008). We calculated total brightness (B) as R300 700, which is the summed reflectance from 300 nm to 700 nm. We also divided our reflectance data into four bins of 100 nm each, calculated the total brightness for each bin (Br=600-700, By=500-600, Bg=400-500, and Bb=300–400), and then calculated chroma: $\sqrt{(BrBg)^2+(ByBb)^2}$ and hue: arctan[(ByBb)/B]/[(BrBg)/B].

Molecular markers.—A subset of the crickets in our dataset was previously genotyped for mitochondrial DNA haplotype (N = 1,132, Harrison et al. 1987, Harrison and Bogdanowicz 1997, Willett et al. 1997, Maroja et al. 2009a, Larson et al. 2013b) and/or 110 Single Nucleotide Polymorphisms (SNPs) from nuclear genes with elevated divergence between G. pennsylvanicus and G. firmus (N = 559, Larson et al. 2013a, 2014). Mitochondrial DNA haplotype was determined by sequencing cytochrome c oxidase I, the adjacent tRNA-Leu, and a portion of cytochrome c oxidase II (Harrison et al. 1987, Willett et al. 1997). SNPs were identified from transcriptomes of male accessory glands from two focal populations (Ithaca, NY and Guilford, CT; Andrés et al. 2013) were genotyped using the Sequenom MassARRAY platform (Larson et al. 2013a, 2014). We used these genotype data to recalculate the hybrid index while accounting for hemizygosity for male X-linked markers using the methods from Shastry et al. (2021). This was especially important because nearly half of these 110 SNPs are located on the X chromosome (Maroja et al. 2015, Gainey et al. 2018). We defined the hybrid index as the proportion of alleles that were inherited from *G. firmus* (hybrid index = 1; Guildford, CT (GUI); Tom's River, NJ (TOM); and Parksley, MD (MET, a.k.a. PAR in Larson et al. 2013a, 2014) and *G. pennsylvanicus* (hybrid index = 0; Ithaca, NY (ITH); Scranton, PA (SCR); State College, PA (SCO)).

Analysis of morphological traits and molecular markers.—All analyses were conducted in R v4.1.2 (R Core Team 2020). To manipulate the data, we used the R packages *dplyr* v1.0.6 and *tidyverse* v1.3.1. To plot our data, we used the R packages *ggplot2* v3.3.5 and *ggpubr* v0.4.0, and to make our maps, we used *Maps* v3.3.0. For statistical analyses, we used commands from the R packages *MASS* v7.3-54 and *car* v3.0-12. We used the R packages *corrplot* v0.92 and *Hmisc* v4.5-0 to determine environmental variable correlation. We used the R packages *AICcmodavg* v2.3-1 and *MnMln* 1.43.1 to rank models based on Akaike Information Criterion and test models.

To test for differences in morphological traits between species and regions, we used the Kruskal-Wallis test followed by a pairwise Wilcoxon rank sum test (PWRST) to determine differences between multiple groups. We chose these non-parametric tests because our dataset failed Levene's test for homogeneity of variance. We quantified how well morphological traits could classify crickets using a linear discriminant analysis (LDA) on allopatric crickets. For all analyses, we present the unadjusted p-values and indicate in bold the values that were significant following FDR correction (Benjamini and Hochberg 1995).

Environmental predictors of species distributions.—We tested the relationships between phenotype and environmental variables that we predicted would be important in determining species range or local adaptation on two scales: 1) across species ranges and 2) at an intermediate scale in a well-characterized region of the hybrid zone (Connecticut). Across the species ranges, we used only allopatric crickets that were most clearly differentiated by morphology, and at the intermediate scale, we used both allopatric and sympatric crickets. We focused on the two phenotypes that best distinguished the two species and that were quantified in most of our samples: ovipositor length and pronotum width.

We identified 10 environmental variables that might be good predictors of species' distributions based on the natural history of these species and prior studies of the field cricket hybrid zone (longitude, latitude, elevation, precipitation, minimum temperature, maximum temperature, human footprint, and three soil characteristics; see Larson et al. 2013b). Elevation, precipitation, and temperature data were collected from the PRISM Climate Group website (https://prism.oregonstate.edu/). Elevation was calculated using an 800-m digital elevation model of the continental United States. For each site, we collected precipitation variables and minimum and maximum temperatures for the year in which each cricket was collected. PRISM data were not available for sites in Canada. Soil data were collected from the USDA STATS-GO2 soil survey (US sites: https://www.nrcs.usda.gov/wps/portal/ nrcs/detail/soils/survey/geo/?cid=nrcs142p2_053629) and the Soil Landscapes of Canada database (Canada sites: https://sis.agr. gc.ca/cansis/nsdb/slc/v3.2/index.html). For a subset of sites in the northeastern United States, we used soil data from ISRIC SoilGrids (Poggio et al. 2021) due to the smaller spatial scale. These data were accessed and compiled using the R package soilDB v2.6.14. We used the following variables: average percent sand, average percent clay, and average percent organic matter. Due to the high

intercorrelation of soil variables confirmed through correlation matrix, we excluded average soil percent silt from further analyses. We also obtained spatial data from the Last of the Wild Global Human Footprint dataset (version 3), consisting of anthropogenic impact measured by population density, land use, and transportation access at a 1-km resolution (Venter et al. 2016, 2018).

We used model selection tests that included these 10 environmental variables to find the combination of variables that best explains morphological variation. We ranked competing models using Akaike Information Criterion (AIC), and we reported the models with the highest goodness-of-fit.

Data accessibility.—All morphological data and collection site information, including GPS coordinates and environmental data and scripts, are published in Dryad (doi:10.5061/dryad.jwstqjqdx).

Results

Estimates of body size.-In total, our dataset comprised 4,221 crickets, with > 1,100 crickets per sex for each morphological trait measured, except for male tegmina color (Table 1). We first evaluated the relationship between three morphological traits that reflect overall body size in crickets: body length, femur length, and pronotum width. We found that body length measurements could vary depending on how crickets responded to being frozen in the lab or other factors such as number of eggs or last meal (see also Weissman and Gray 2019). Consequently, we chose to exclude body length measurements from our analyses but include them in our supplemental datasets. Male and female individuals of both G. pennsylvanicus and G. firmus had strong positive relationships between femur length and pronotum width (male G. pennsylvanicus: $R^2 = 0.53$, $F_{1,233}$ = 265, p < 2.2×10^{-16} and male G. *firmus*: R² = 0.76, F_{1.117} = 363.1, p < 2.2×10⁻¹⁶, Suppl. material 1: fig. S1A; female G. pennsylvanicus: R² = 0.53, $F_{1,192}$ = 21, p < 2.2×10⁻¹⁶ and female G. firmus: R^2 = 0.74, $F_{1,89}$ = 254.7, p < 2.2×10^{-16} , Suppl. material 1: fig. S1B). Therefore, we used pronotum width as our estimate for overall body size to maximize the number of individuals we could compare across datasets.

In female individuals, pronotum width and ovipositor length were also positively related in both species (*G. pennsylvanicus*: $R^2 = 0.44$, $F_{1,214} = 165.7$, $p < 2.2 \times 10^{-16}$ and *G. firmus*: $R^2 = 0.26$, $F_{1,87} = 30.48$, $p = 3.44 \times 10^{-7}$, Suppl. material 1: fig. S1C). In comparisons with *G. thinos*, we used femur length to estimate body size to maximize the number of individuals in those comparisons.

Morphological differences between species.—There were significant differences among allopatric G. pennsylvanicus, G. firmus, G. thinos, and sympatric populations (e.g., G. firmus, G. pennsylvani*cus*, and hybrids) in male body size (Kruskal-Wallis, $\chi^2 = 35.79$, df = 3, p = 8.29×10^{-8}), female body size (Kruskal-Wallis, $\chi^2 = 51.89$, df = 3, p = 3.16×10^{-11}), female ovipositor length (Kruskal-Wallis, χ^2 = 1277.2, df = 3, p < 2.2×10⁻¹⁶), and relative ovipositor length (Kruskal-Wallis, $\chi^2 = 82.10$, df = 3, p < 2.2×10^{-16}). When comparing allopatric G. pennsylvanicus and G. firmus, male pronotum $(p = 2.1 \times 10^{-5}, Fig. 2A)$, female pronotum $(p = 1.4 \times 10^{-11}, Fig. 2B)$, ovipositor length ($p < 2.2 \times 10^{-16}$, Suppl. material 1: fig. S2A), and relative ovipositor length ($p = 2.8 \times 10^{-16}$, Fig. 2C) were all significantly different. However, for each of these traits, there was still considerable overlap between allopatric species. Ovipositor length had the most striking differences between species (Suppl. material 1: fig. S2A), even when controlling for body size (Fig. 2C).

For males, tegmina color alone classified most individuals from allopatric populations as either *G. pennsylvanicus* or *G. firmus* (LDA, misclassification rate of 3%). One of the 24 *G. pennsylvanicus* males was misclassified as *G. firmus*, and zero of the 7 *G. firmus* males were misclassified as *G. pennsylvanicus*. When looking at male body size alone, the misclassification rate was much higher at 23%, with 56 of the 268 *G. pennsylvanicus* males misclassified and 27 of the 90 *G. firmus* males misclassified. There was not enough overlap in body size and tegmina color data to perform these analyses using both variables. For females, body size and relative ovipositor length classified most individuals from allopatric populations as either *G. pennsylvanicus* or *G. firmus* (LDA, misclassified as *G. firmus*. and 17 of the 90 *G. firmus* were misclassified as *G. pennsylvanicus*.

Table 1. Summary of sample sizes for morphological measurements by sex, population type, and region (See Fig. 1 for location information).

	Pronotum Width		Femur Length		Ovipositor	Ovipositor	Ovipositor	Tegmina
	Females	Males	Females	Males	Length	Pronotum Ratio	Femur Ratio	Color
Totals	1203	1263	1134	1213	4047	1174	1110	469
CTR	4	5	4	5	12	4	4	-
allopatric	4	5	4	5	4	4	4	-
sympatric	-	-	-		8	-	-	-
NE	993	1010	871	849	3739	969	851	449
allopatric	111	167	85	132	1480	108	82	23
sympatric	882	843	786	717	2259	861	769	426
NW	26	17	27	15	27	26	27	-
allopatric	26	17	27	15	27	26	27	-
SE	66	65	77	60	111	62	74	20
allopatric	66	65	77	60	89	62	74	8
sympatric	-	-	-	-	22	-	-	12
SO	40	69	66	171	70	40	66	-
allopatric	40	69	65	171	69	40	65	-
sympatric	-	-	1		1	-	1	-
SW	29	41	29	52	29	29	29	-
allopatric	29	41	29	52	29	29	29	-
WNC	45	56	60	61	59	44	59	-
allopatric	45	56	60	61	59	44	59	-



Fig. 2. Allopatric populations of *G. firmus* and *G. pennsylvanicus* differ in overall body size and ovipositor length. A. Male pronotum width by species; **B.** Female pronotum width by species; **C.** Relative ovipositor length (ovipositor length/pronotum width). Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups (PWRST with corrected p-values < 0.05).

Crickets from areas near the hybrid zone, which we refer to as sympatric, had considerable overlap with those from allopatric populations. Sympatric crickets were not different from *G. firmus* for male body size, but they were, on average, larger than *G. pennsylvanicus* (*G. pennsylvanicus*: $p = 6.0 \times 10^{-6}$, *G. firmus*: p = 0.16, Fig. 2A) but were still different from both allopatric species for female body size (*G. pennsylvanicus*: $p = 9.4 \times 10^{-7}$, *G. firmus*: p = 0.00032, Fig. 2B), female ovipositor length (*G. pennsylvanicus*: $p < 2.0 \times 10^{-16}$, *G. firmus*: $p < 2.0 \times 10^{-16}$, Suppl. material 1: fig. S2A), and female relative ovipositor length (*G. pennsylvanicus*: $p = 4.6 \times 10^{-8}$, *G. firmus*: $p = 1.0 \times 10^{-9}$, Fig. 2C). This suggests that while these sympatric populations may have individuals that are more *G. firmus*-like or *G. pennsylvanicus*-like, they still have intermediate morphology compared to allopatric populations.

Intraspecific variation in key morphological traits.-We then tested how these traits varied across the different geographic regions of each species. We found differences among regions of G. pennsylvanicus for male pronotum (Kruskal-Wallis, $\chi^2 = 56.11$, df = 6, p = 2.76×10^{-10}), female pronotum (Kruskal-Wallis, χ^2 = 63.44, df = 6, p = 8.9×10^{-12}), ovipositor length (Kruskal-Wallis, χ^2 = 185.72, df = 6, $p < 2.2 \times 10^{-16}$), and relative ovipositor length (Kruskal-Wallis, χ^2 = 33.6, df = 6, p = 8.03×10⁻⁶). Male and female *G. pennsylvanicus* were largest in the southern and midcentral US (SE, SO, SW, CTR, Fig. 3A, B), and they had the smallest body size in the northern west (WNC, NW). There were differences among regions in G. firmus pronotum width (Kruskal-Wallis, males, $\chi^2 = 9.27$, df = 2, p = 0.01; females, χ^2 = 9.15, df = 2, p = 0.01), in ovipositor length (Kruskal–Wallis, $\chi^2 = 78.65$, df = 2, p < 2.2×10^{-16}), and relative ovipositor lengths (Kruskal-Wallis, $\chi^2 = 54.49$, df = 2, p = 1.47×10^{-10} ¹²). Male and female G. firmus were larger in the south than in the northeast, while G. firmus in the south were not significantly different from crickets in either the northeast or the southeast (Fig. 3A, B). In G. pennsylvanicus, ovipositor length varied by region. Eastern populations (NE, SE) had the shortest ovipositors, and those from the central US (CTR) had the longest ovipositors, although there was a very limited sample size for this region (Suppl. material 1: figs S2B, S3C). There was considerable variation in ovipositor length among G. firmus populations; southern G. firmus females had significantly shorter relative ovipositors than G. firmus in the southeast, who in turn had shorter relative ovipositors than G. firmus in the northeast (Suppl. material 1: figs S2C, S3C). However, G. firmus in the southeast had very similar absolute ovipositor lengths to northeastern G. firmus but had larger body sizes,

whereas southern *G. firmus* simply had shorter ovipositors (Suppl. material 1: fig. S2C).

Recent work by Weissman and Gray (2019) documented cryptic variation in southern USA G. firmus, so we took a closer look at these populations, separating crickets collected in Florida from those collected in Texas. We also included the recently described closely related species G. thinos, which is sympatric with Texas G. firmus (Weissman and Gray 2019). We found that male (Kruskal–Wallis, $\chi^2 = 29.26$, df = 3, p = 1.98×10^{-6}) and female (Kruskal-Wallis, $\chi^2 = 24.88$, DF = 3, p = 1.63×10^{-5}) body size and ovipositor length (ovipositor length: Kruskal-Wallis, $\chi^2 = 101.39$, df = 3, p < 2.2×10^{-16} ; relative ovipositor: Kruskal-Wallis, χ^2 = 89.57, df = 3, p < 2.2×10^{-16}) differ among these groups (Fig. 4, Suppl. material 1: fig. S2). Compared to northeastern G. firmus, Florida G. firmus were much larger (Fig. 4A, B) but had only slightly larger ovipositor lengths (Suppl. material 1: fig. S2C), giving them shorter relative ovipositors (Fig. 4C). Texas G. firmus did not differ in overall body size from northeastern G. firmus but had even shorter relative ovipositor lengths (Fig. 4C, Suppl. material 1: fig. S2C). The magnitude of the morphological differences among Florida, Texas, and northeastern G. firmus is similar to that of the differences between G. firmus and the recently described G. thinos. Gray et al. (2020) found that G. firmus in Texas and Florida are genetically distinct groups, with Texas G. firmus sister to G. pennsylvanicus and Florida G. firmus sister to both G. pennsylvanicus and Texas G. firmus. Altogether, the morphological differences and phylogenetic relationships support the findings by Weissman and Gray (2019) that Texas G. firmus may be an undescribed cryptic species.

Morphology in sympatric populations.—For the subset of crickets that were from the hybrid zone or nearby (sympatric populations) and were also genotyped with molecular markers, we looked at the relationship between admixture and morphological traits. We found that each trait had a similar transition from *G. pennsylvanicus* to *G. firmus*, with highly admixed individuals having intermediate phenotypes (Fig. 5). We found that male pronotum (R² = 0.19, $F_{1,279} = 63.35$, p = 4.38×10^{-14}), male tegmina color (R² = 0.31, $F_{1,133} = 60.82$, p = 1.62×10^{-12}), female pronotum (R² = 0.28, $F_{1,275} = 107.3$, p < 2.2×10^{-16}), and relative ovipositor length (R² = 0.47, $F_{1,270} = 243.1$, p < 2.2×10^{-16}) all had strong correlation with the hybrid index. Because the SNPs used to calculate the hybrid index are concentrated on the X chromosome (54 out of 110 (Maroja et al. 2015), Gainey et al. 2018)), females (XX) were more likely to be classified with an intermediate hybrid index than males (XO). Overall,



Fig. 3. Cricket body size and relative ovipositor length varies by geographic region. **A.** Male pronotum width by species and region; **B.** Female pronotum width by species and region; **C.** Relative ovipositor length by species and region. Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups within each species (PWRST with corrected p-values < 0.05), and exact p-values are presented in Suppl. material 1: tables S1, S2. See Fig. 1 for location information.

morphological traits were also correlated with mtDNA haplotypes: crickets that had *G. pennsylvanicus* mtDNA tended to be smaller (males: Kruskal-Wallis, $\chi^2 = 43.14$, df = 1, p = 5.11×10^{-11} ; females: Kruskal-Wallis, $\chi^2 = 44.86$, df = 1, p = 2.11×10^{-11}), darker (Kruskal-Wallis, $\chi^2 = 33.75$, df = 1, p = 6.27×10^{-9}) crickets with shorter ovipositors (Kruskal-Wallis, $\chi^2 = 37.67$, df = 1, p = 8.40×10^{-10}) (Fig. 6). We found that crickets with *G. firmus* ancestry at nuclear markers (hybrid index = 1) often had *G. pennsylvanicus* mtDNA haplotypes (Fig. 7), indicating asymmetric introgression of the mtDNA.

Environmental predictors of morphology.—In allopatric populations throughout broad ranges, we found that latitude, elevation, average

soil percent clay, and minimum and maximum temperatures created the best model for ovipositor length. Latitude, longitude, soil percent sand, and minimum temperature created the best model for pronotum width (Table 2). Average soil percent clay and higher minimum and maximum temperatures were positively associated with longer ovipositor lengths, and higher minimum temperatures were positively associated with larger body size, which are characteristics of *G. firmus* (Suppl. material 1: fig. S3). In the subset of Connecticut sympatric and allopatric populations, minimum and maximum temperatures, as well as soil percent organic matter, created the best model, with positive associations for all three variables and ovipositor length (Table 2, Suppl. material 1: fig. S3).



Fig. 4. Morphological variation in *G. firmus* consistent with proposed cryptic species. **A.** Male femur length; **B.** Female femur length; **C.** Relative ovipositor length (ovipositor length/femur length). There is considerable morphological variation among northeastern, Florida, and Texas *G. firmus*, which is similar to the magnitude of morphological divergence observed in the closely related species *G. thinos*. This combined with genetic divergence suggests there may be cryptic species in what is currently considered *G. firmus*. Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups (PWRST with corrected p-values < 0.05), and exact p-values are presented in Suppl. material 1: table S3.



Fig. 5. Crickets with more hybrid background have intermediate morphological traits. The relationship between the hybrid index (an estimate of ancestry proportions, *G. pennsylvanicus* = 0 and *G. firmus* = 1) and **A.** Male pronotum width; **B.** Female pronotum width; **C.** Relative ovipositor length, and **D.** Male tegmina color.



Fig. 6. Morphological traits tended to correspond to mtDNA haplotypes. A. Male pronotum width; B. Female pronotum width; C. Relative ovipositor length; and D. Male tegmina color. Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots.

Table 2. Results of linear regression and AIC to test the relationship between environmental variables and morphological traits in female crickets of both species. ¹ indicates variables where values are based on the year the samples were collected.

A. Ovipositor length												
	Df	Sum of Sq	RSS	AIC	Coefficient	St. Error	t-value	p-value				
(Intercept)		-	887.86	321.96	16.213	0.132	122.417	< 2.00E-16				
Latitude	1	9.283	897.14	322.33	0.545	0.358	1.523	0.129				
Precipitation ¹	1	1.054	886.81	323.69	-	-	-	-				
Longitude	1	0.389	887.47	323.86	-	-	-	-				
Human Footprint	1	0.132	887.73	323.93	-	-	-	-				
Avg Soil % Sand	1	0.015	887.85	323.96	-	-	-	-				
Avg Soil % Organic Matter	1	0.004	887.86	323.96	-	-	-	-				
Elevation	1	26.035	913.90	326.55	-0.638	0.250	-2.551	0.011				
Avg Soil % Clay	1	26.562	914.42	326.68	0.365	0.142	2.577	0.011				
Minimum Temperature ¹	1	29.311	917.17	327.36	-1.244	0.459	-2.707	0.007				
Maximum Temperature ¹	1	124.629	1012.49	349.91	2.281	0.409	5.582	6.89E-08				
			B. Proi	notum width								
(Intercept)		-	36.539	-253.9	5.83503	0.036	162.636	< 2.00E-16				
Maximum Temperature ¹	1	0.381	36.158	-253.69	-	-	-	-				
Precipitation ¹	1	0.176	36.363	-252.73	-	-	-	-				
Human Footprint	1	0.147	36.392	-252.59	-	-	-	-				
Elevation	1	0.103	36.436	-252.38	-	-	-	-				
Avg Soil % Clay	1	0.088	36.451	-252.31	-	-	-	-				
Avg Soil % Organic Matter	1	0.013	36.526	-251.96	-	-	-	-				
Minimum Temperature ¹	1	2.964	39.503	-242.56	-0.233	0.064	-3.670	3.27E-04				
Avg Soil % Sand	1	3.953	40.492	-238.33	-0.180	0.043	-4.238	3.73E-05				
Longitude	1	4.618	41.157	-235.55	-0.187	0.041	-4.580	9.07E-06				
Latitude	1	12.890	49.429	-204.23	-0.536	0.070	-7.652	1.53E-12				

Discussion

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Cryptic diversity in a wide-ranging species.—The hybrid zone between the field crickets *G. firmus* and *G. pennsylvanicus* has been a model for understanding speciation (Harrison and Rand 1989, Harrison and Larson 2014). The field cricket hybrid zone stretches from the northeastern United States as far south as Virginia and likely farther into the southeast. Divergence in morphology, nuclear and mitochondrial DNA, and reproductive barriers have been carefully studied in several major regions of the hybrid zone (Harrison 1985, Rand and Harrison 1989, Ross and Harrison 2002, Maroja et al. 2009a, 2009b, Larson et al. 2012, 2014). Yet, even in this well-studied system, there is geographic diversity across the ranges of these species that complicates their relationships.

Our results confirm that allopatric populations of these two species, defined by genetic markers (Harrison and Arnold 1982, Willett et al. 1997, Broughton and Harrison 2003, Maroja et al. 2009a), can be largely differentiated by a combination of body size, male tegmina color, and female ovipositor length (Fig. 2). At the same time, there is regional variation in these traits within each species (Fig. 3). These differences may be due to local adaptation of life history traits such as egg diapause and development time (discussed below) or phenotypic plasticity. However, in some cases, they may also indicate cryptic diversity in field crickets.

In their revision of North American field crickets, Weissman and Gray (2019) proposed that there was cryptic diversity in the southern populations of *G. firmus*, particularly in Texas. Importantly, our phenotypic comparisons confirmed that Texas and Florida *G. firmus* are morphologically distinct from northeastern *G. firmus* (Fig. 4). In a recent nuclear phylogeny, Texas and Florida *G. firmus*-like crickets also formed distinct clusters within the larger *G. pennsylvanicus* group (Weissman and Gray 2019, Gray et al. 2020). Unfortunately, we do not have a phylogeny that includes



Fig. 7. Mitochondrial DNA introgression is largely asymmetric. Crickets with *G. firmus* ancestry at nuclear markers (hybrid index = 1) often had *G. pennsylvanicus* mtDNA haplotypes.

genes from both Texas and Florida *G. firmus* and northeastern *G. firmus*, so the relationships among these groups are still unclear. However, the combination of distinct morphology and phylogenetic relationships suggests that at least one cryptic species of *Gryllus* exists, a situation that will not be resolved without further genotyping and/or evaluations of reproductive compatibility among these populations.

Intermediate phenotypes in hybrid zone crickets.—The morphological traits that best distinguish species in allopatry can also be used to distinguish these species in or near the hybrid zone. In this study, we took a conservative approach to defining allopatric and sympatric populations. Allopatric populations were those well outside of where the two species co-occur and are typically populations that have been genotyped with speciesdiagnostic markers. We found that in sympatry, crickets that were mostly G. firmus or mostly G. pennsylvanicus at nuclear markers (Larson et al. 2013a, 2014) had morphological traits that are also G. firmus-like or G. pennsylvanicus-like. Both male and female body size, male tegmina color, and relative ovipositor length had clinal variations from G. pennsylvanicus-like to G. firmus-like, with highly admixed individuals having intermediate phenotypes (Fig. 5). Male tegmina color stood out as having the fewest individuals with intermediate hybrid index values (Fig. 5D), but this is most likely because the SNPs used to calculate the hybrid index were predominately X-linked, so male XO crickets were rarely heterozygous at those SNPs and had overall lower hybrid indices (Larson et al. 2014, Maroja et al. 2015, Gainey et al. 2018).

The relationship between morphology and mitochondrial haplotype was less clear for populations near or in the hybrid zone. Crickets that were mostly *G. firmus* at the nuclear markers often had *G. pennsylvanicus* mtDNA (Fig. 7). This pattern fits with what we expect based on the one-way prezygotic incompatibility between *G. firmus* females and *G. pennsylvanicus* males (Harrison 1983, Maroja et al. 2009b, Larson et al. 2012). All F1 hybrids are produced from crosses with *G. pennsylvanicus* mothers; thus, *G. pennsylvanicus* mtDNA will be more likely to introgress into *G. firmus*. Even rare instances of hybridization might lead to mtDNA introgression, such as the mtDNA capture observed in many mammal species (Melo-Ferreira et al. 2005, Good et al. 2008).

Adaptations to soil type.—Ovipositor length is one of the most striking morphological differences between G. firmus and G. pennsylvanicus. Female crickets use their ovipositors to lay their eggs in the soil, and ovipositor length has been hypothesized to relate to the soil type and/or the depth of egg laying (Masaki 1979). The depth of egg laying may be a particularly critical life-history trait in G. pennsylvanicus and G. firmus because these species overwinter as eggs, as opposed to most field crickets that overwinter as early instar nymphs (Alexander 1968, Harrison and Bogdanowicz 1995). For eggs to be viable, they must withstand low winter temperatures and freeze/thaw cycles (Ross and Harrison 2006). Throughout its range, G. firmus is most often found on sandy coastal soils (Harrison and Arnold 1982, Weissman and Gray 2019) and tends to have a longer ovipositor than G. pennsylvanicus (Fig. 3, Suppl. material 1: fig. S2). This may be an adaptation to laying eggs deeper in sandy substrates in response to intermittent rainfall and the risk of eggs drying out (Walker 1980). In some parts of the hybrid zone, such as Connecticut, the association with different soil types is striking. The two species have been found on micro habitat patches of loam (G. pennsylvanicus) and sandy (G. firmus) soils in Connecticut (Harrison 1986, Harrison and Rand 1989, Rand and Harrison 1989), and interactions between the two species occur across these habitat patch boundaries on a scale of only hundreds of meters (Ross and Harrison 2002, Larson et al. 2014).

Despite what appears to be strong habitat associations, the relationship between soil type and ovipositor length is complicated. Ovipositor length does not necessarily determine egg-laying depth; instead, females may wield long ovipositors at different angles (Réale and Roff 2002). It is also not clear exactly how the association between ovipositor length and soil type is maintained. Females of both species prefer to lay eggs in loamy soil, and there is no difference in overwintering egg viability in different soil types (Ross and Harrison 2006). Finally, these associations are clearly established only in a small part of the species' ranges, i.e., Connecticut (Rand and Harrison 1989, Ross and Harrison 2002, Larson et al. 2013b). Even where soil associations appear to be the strongest, the transition from sandy to loamy soils is more gradual and less distinct than we might expect based on the patchiness of G. firmus and G. pennsylvanicus populations (Ross and Harrison 2002, Larson et al. 2014). Here we find that both across the broad ranges of these species and at an intermediate scale in the Connecticut hybrid zone, there is no strong association between ovipositor length and sandy soils. In fact, we tend to see crickets with longer ovipositors on clay soils (Table 2). This might be due to the different methods used to quantify soil type (soil survey data versus on-site soil sampling), but altogether, this suggests that habitat associations in these species are variable and should be investigated further.

Body size, climate, and life cycle.—In insects, seasonality and the length of the growing season are critical to the rate of development and adult body size (Masaki 1961, Tauber and Tauber 1981). This is particularly true for hemimetabolous insects, which often go through many nymphal stages and have long development times before reaching their full size and sexual maturity (Kivelä et al. 2011). Insects at higher latitudes have shorter growing seasons and, as a result, may develop more quickly or reach an overall smaller body size (Masaki 1967, Parsons and Joern 2014). This pattern of smaller body sizes at higher latitudes is sometimes referred to as the converse of Bergman's rule, which states that individuals have larger body sizes in colder climes (Masaki 1967, Mousseau 1997). We see this pattern most clearly in *G. pennsylvanicus*, where **Acknowledgements** we found that populations with the smallest body sizes tended to be farther north (WNC and NW, Fig. 2). Indeed, we found that crickets at higher latitudes had, on average, smaller body sizes and that there was a significant relationship between body size and latitude (Table 2).

We may not expect a direct relationship between body size and latitude if the length of the growing season allows for multiple generations per year. Insects can shift from continuous development in the south to univoltine (one generation per year) in the north (Masaki 1961, 1967). As a result, there may be regions where body size is smaller than expected based on latitude to accommodate multiple generations per year. We did not find this pattern in our results, but we may not have had the resolution of latitudinal samples to see a sawtooth pattern in body size. However, there is some evidence that development time in G. firmus varies with latitude. In Virginia, G. firmus emerge earlier in the season than G. pennsylvanicus, leading to temporal isolation in that part of the hybrid zone, but in Connecticut, the two emerge simultaneously (Harrison 1985). In Florida, G. firmus is reported to have multiple generations per year (Walker, personal observation; reported in Weissman and Gray 2019), where throughout its range, it otherwise appears to have a single generation per year (Walker 1980). Notably, despite having many generations per year, Florida G. firmus are considerably larger than northern populations. It is unclear whether there is a continuous shift in life cycle across the range of G. firmus or if Florida G. firmus have a distinct life history from other G. firmus.

Conclusions

In studies of speciation and to understand the effects of local selection, it is critical to quantify morphological and genetic variations across the geographic range of widespread species. The field cricket hybrid zone is an example of how important the larger geographic context can be. In some regions of the field cricket hybrid zone, G. pennsylvanicus and G. firmus have a patchy distribution, and G. firmus crickets are found on sandy soils (Rand and Harrison 1989, Ross and Harrison 2002). However, the strong soil association breaks down in other regions of the hybrid zone (Larson et al. 2013b) and across their geographic range, suggesting that the soil association may be a result of local adaptation or colonization history (Hauffe and Searle 1993, Gompert et al. 2010). Our results provide a foundation for future geographically expansive studies that compare genetic divergence and the role of specific traits in reproductive barriers to better understand local adaptation and speciation in this system. More broadly, this is an example of how critical it is to move studies of speciation beyond the comparison of a few focal populations. Geographically expansive studies of phenotypic and genetic divergence will also be important for understanding how species distributions and hybrid zones shift over time and in a changing climate (Britch and Cain 2001, Taylor et al. 2015).

Author Contributions

ARB, CJ, DBW, DAG, CLR, LSM and ELL collected the data; ARB, CJ, and ELL combined the datasets; AG obtained the environmental data and advised on analyses. ARB conducted all analyses. ARB and ELL wrote the manuscript with contributions from all authors.

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- Explanation note: table S1. P-values for PWRST posthoc contrasts of allopatric G. pennsylvanicus populations by region (See Fig. 3). Pvalues marked with *** lost significance after correction. table S2. P-values for PWRST posthoc contrasts of allopatric G. firmus populations by region (See Fig. 3). P-values marked with *** lost significance after correction. table S3. P-values for PWRST posthoc contrasts of G. thinos populations and G. firmus populations in the Northeast, Florida, and Texas. See Fig. 4. figure S1. Relationship among phenotypic characteristics for allopatric populations. (a) Male Gryllus firmus (yellow) and G. pennsylvanicus (teal) linear models show positive relationships in femur length and pronotum width (*G. firmus*: R2 = 0.76, F1, 117 = 363.1, p-value < 2.2e-16; G. pennsylvanicus: R2 = 0.53, F1,233 = 265.0, p-value < 2.2e-16). Female linear models show positive relationships in both (b) femur length and pronotum width (G. firmus: R2 = 0.74, F1,89 = 254.7,p-value < 2.2e-16; G. pennsylvanicus: R2 = 0.53, F1,192 = 217.0, p-value < 2.2e-16) and (c) ovipositor length and pronotum width (G. firmus: R2 = 0.26, F1,87 = 30.48, p-value = 3.44e-07; G. pennsylvanicus: R2 = 0.44, F1,214 = 165.7, p-value < 2.2e-16). figure S2. Ovipositor length differences between species and among populations of each species. A. Ovipositor length differences between G. firmus, G. pennsylvanicus and sympatric populations (G. firmus vs G. pennsylvanicus p < 2.0E-16; G. firmus vs sympatric p <2.0E-16; G. pennsylvanicus vs sympatric p <2.0E-16). B. Ovipositor length differences among populations of G. pennsylvanicus. Posthoc p-values are presented in Suppl. material 1: table S1. C. Ovipositor length differences among populations of G. firmus and G. thinos. Posthoc p-values are presented in Suppl. material 1: table S3. Boxplots indicate the mean values of each trait, quartiles and the range of the data (whiskers). Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups (PWRST with corrected p-values < 0.05). figure S3. Scatterplots of significant AIC environmental variables. For all female allopatric populations: ovipositor length vs. latitude (a.), elevation (b.), average soil percent clay (c.), minimum temperature (d.), and maximum temperature (e.). For all female allopatric populations: pronotum width vs. latitude (f.), longitude (g.), average soil % sand (h.), and minimum temperature (i.). For female allopatric and sympatric Connecticut populations: ovipositor length vs. minimum temperature (j.), maximum temperature (k.), and average soil % organic matter (l.).
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