The phylogenetic placement of the rare North American band-winged grasshopper *Shotwellia isleta* Gurney, 1940 (Orthoptera: Acrididae: Oedipodinae)

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Abstract
The phylogenetic placement of the North American band-winged grasshopper *Shotwellia isleta* (Gurney 1940) (Orthoptera: Acrididae: Oedipodinae) has been investigated. This rare and unique species of grasshopper belongs to a monotypic genus known only from a few isolated locations in the Chihuahuan Desert of Mexico and New Mexico, USA. The taxonomic status of *Shotwellia* has been uncertain and historically classified in very different ways relative to other genera. We conducted the first molecular phylogenetic cladistic analysis of *Shotwellia* and of the *Chortophaga* (Saussure) genus group in which it is currently classified, using three mitochondrial genes (16S, 12S and cytochrome c oxidase II), 14 morphological characters and two behavioral characters. Our analysis supports the current monotypic status of the genus *Shotwellia* within the *Chortophaga* genus group and indicates that *Shotwellia* is sister to the other genera in that group. *Shotwellia isleta* is a species of conservation concern, and our field sampling indicates that it is a rare specialist of freshwater ephemeral desert lake beds that are being impacted by human activity. Understanding the phylogeny of *S. isleta* provides a basis from which biological, evolutionary and conservation inferences can be made.

Keywords
Phylogenetics, molecular evolution, *Encoptolophus, Chortophaga, Arphia*

Introduction
*Shotwellia isleta* (Gurney 1940; Figs 1 and 2) is a rare band-winged grasshopper (Orthoptera: Acrididae: Oedipodinae) in a monotypic genus endemic to the Chihuahuan Desert of North America. *Shotwellia* is known from only a few localities, and only six collection records have been reported in the literature since the initial description of the taxon in 1940 (Gurney 1940; Otte 1984). Originally described from New Mexico, USA, the species is also known from a few isolated locations in the states
of Durango and Zacatecas, Mexico (Fig. 3). *Shotwellia isleta* is currently listed as an imperiled genus and species of conservation concern because of its rarity in nature (Nature Serve 2009). In addition to its relative rarity, the species apparently occurs in only fresh-water ephemeral desert lake beds (Fig. 4). These ephemeral habitats support a multitude of other plant and animal species not present in the surrounding desert habitats, and probably play an important role in local biodiversity.

*Shotwellia isleta* was originally described by Gurney (1940), who considered the genus to be most closely related to the genus *Hadrotettix* Scudder, a member of the currently recognized *Hippiscus* Saussure genus group of Otte (1984) (=Hippiscini Eades et al., 2009). Gurney (1940) classified the genus near *Hadrotettix* and *Anconia* Scudder based upon the low median pronotal carina in all three genera and similarities in the hind legs and ovipositor. More recently, Otte (1984) placed *Shotwellia* in the *Chortophaga* group (=Chortophagini Eades et al., 2009) based largely on the similar
morphology of the male aedeagus to *Encoptolophus* Scudder and *Chortophaga* species. Other characters that Otte (1984) considered to be important in classifying *S. isleta* included; (1) the single large medial dark band on the forewing (Fig. 2), (2) absence of a second basal dark tegminal band above the base of the hind femora when viewed laterally (Fig. 1), (3) absence of dark bands on the medial area of the hind femora (Fig. 1), (4) absence of a median pronotal ridge (Fig. 1) and (5) yellow hind tibiae.

The most recent and comprehensive phylogenetic construction of the *Chortophaga* group by Otte (1984) hypothesized *Shotwellia* to be sister to *Encoptolophus*, and *Chortophaga* + *Chimarocephala* to be sister to *Shotwellia* + *Encoptolophus*. The only molecular phylogenetic studies on North American band-winged grasshoppers to date (Chapco et al. 1997; Fries et al. 2007) examined only a few distantly related taxa, and did not include *Shotwellia*, but these analyses are not consistent with those of Otte (1984). Striking differences between phylogenetic hypotheses of these groups by Gurney (1940) and Otte (1984), along with a lack of comprehensive modern analysis of these groups, suggest a need for a modern cladistic analysis to better place *Shotwellia* and determine relationships between other members of the *Chortophaga* and *Hippiscus* and genus groups. Fries et al. (2007) further demonstrated that the *Chortophaga* genus group is monophyletic, and much older than most world Oedipodinae and the rest of North American band-winged grasshoppers, which form another more recent monophyletic clade. However, they did not include *Shotwellia* in their analysis.

The purpose of this project is to conduct a combined phylogenetic analysis based on molecular, morphological and behavioral data to determine relationships of *Shotwellia* to other taxa within the *Chortophaga* genus group, and to some members of the *Hippiscus* genus group to address Gurney’s original classification, and also to the *Arphia* genus group which is closely related to the *Chortophaga* genus group (Otte 1984). An understanding of the phylogeny of this unusual and rare taxon will shed light on the evolution of its unusual habitat requirements, behaviors and other aspects of its ecology, and these are discussed briefly.

Very little systematic research has been conducted on North American band-winged grasshoppers involving cladistics and DNA analysis. Chapco et al. (1997) and Fries et al. (2007) are the only studies, and those works include few genera and species. The most widely accepted current classification of these grasshoppers is that by Otte (1984). Although Otte (1984) presents hypothesized phylogenies for genus groups and species, those phylogenies are based upon his authoritative comparisons of morphological characters outside the context of formal cladistic analyses. Therefore, this analysis represents one of the first combined molecular and morphological character analyses in the subfamily.

**Materials and Methods**

**Taxon sampling**

Twenty-six specimens representing 22 species (Table 1) were selected for analysis. Specimens were collected by D.C.L., W.C.E. and D.B. Weissman (Department of Entomology, California Academy of Sciences), and were identified by D.C.L. Voucher
Table 1. Taxa used in analysis including locality data, GenBank accession numbers for DNA sequences and UNM voucher numbers.

<table>
<thead>
<tr>
<th>Genus group</th>
<th>Species</th>
<th>Collection information</th>
<th>GenBank accession No. (12S,16S, COII)</th>
<th>UNM voucher No. (MSBA, Miller Lab)</th>
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<tr>
<td>Arphia</td>
<td>Arphia conspersa</td>
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<td>Arphia</td>
<td>L. elota</td>
<td>MEXICO. Jalisco, 43 km W, Guadalajara, km 151 on Hwy. 15, 20°53′19″ N 103°56′12″ W, 31 May 2008, D.C.Lightfoot, D.B.Weissman, coll. DNA08-4</td>
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<td>L. punctatus</td>
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<td><em>Arphia</em></td>
<td><em>Tomonotus ferruginosus</em></td>
<td>USA. Arizona, Chiricahua Mts., Cave Creek Canyon, 31°52’43” N 109°13’21” W, 21 Jun 2008, W.C.Edelman, D.C.Lightfoot, coll. DNA08-44</td>
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<td><em>T. mexicanus</em></td>
<td>MEXICO. Michoacan, km 224 on Hwy. 15., 19°41’17” N 101°01’02” W, 02 Jun 2008, D.C.Lightfoot, D.B.Weissman, coll. DNA08-20</td>
<td>GU476936, GU476961, GU476983</td>
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<td><em>Chimarocephala elongata</em></td>
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<td>USA. New Mexico, Socorro Co., nr. Bernardo, Hwy. 60, 34°25’01” N 106°47’49” W, 11 May 2005, D.C.Lightfoot, coll. DNA05-20</td>
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<td><em>Encoptolophus fuliginosus</em></td>
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<td><em>E. subgracilis</em></td>
<td>USA. New Mexico, Dona Ana Co., Lake Isacs, nr. Las Crucens, 32°27’38” N 106°43’12” W, 04 Oct 2004, D.C.Lightfoot, coll. DNA04-352</td>
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<th>UNM voucher No.</th>
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<td>Chortophaga</td>
<td>Shotwellia isleta</td>
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<td>Hippiscus</td>
<td>X. montanus</td>
<td>USA. New Mexico, Socorro Co., nr. Bingham, Hwy. 280, 33°52’50” N 106°31’43” W, 22 May 2005, D.C.Lightfoot, coll. DNA05-17</td>
<td>GU476941, GU476970, GU476986</td>
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</table>

* Samples which did not amplify for the specified gene.
DNA sequences

Whole metathoracic or mesothoracic legs were removed from each specimen immediately after collection in the field and preserved in 95% ethanol. These legs were then frozen until DNA extraction. DNA was extracted from large legs by removing and extracting muscle tissue. If legs were small, the entire leg was extracted. DNA was extracted using the DNEasy blood and tissue kit (Qiagen, Valencia, CA, USA) and the animal tissue protocol.

Three genes were used in the analysis: 16S rRNA (16S), 12S rRNA (12S) and cytochrome c oxidase subunit II (COII). A preliminary screening of genes across several Oedipodinae genera revealed that the genes histone III and 28S rRNA were identical, even across distantly related genera. Therefore, they, and additional nuclear genes, were not pursued as potential markers for this project. Each gene was amplified for all specimens except for several which either failed to amplify or sequence (Table 1). PCR and sequencing protocols closely followed Miller et al. (2007). DNA fragments were amplified using PCR with TaKaRa Ex Taq (Takara Bio, Otsu, Shiga, Japan) on an Eppendorf Mastercycler ep gradient S Thermal Cycler (Eppendorf, Hamburg, Germany) and visualized using gel electrophoresis. PCR purification was done using ExoSAP-IT USB-Affymetrix, Cleveland, OH, USA) and cycle sequenced using ABI Prism Big Dye (version 3.1, ABI, Fairfax, VA, USA) using the same primers used for amplification. Sequencing reaction products were purified using Sephadex G-50 Fine (GE Healthcare, Uppsala, Sweden) and sequenced with an ABI 3130xl Genetic analyzer (Molecular Biology Facility, UNM). All gene regions were sequenced in both directions, and sequences were edited using Sequencher (Genecodes 2006).

According to Song et al. (2008), nuclear mitochondrial pseudogenes (numts) are a concern that threatens the assumption of orthology, especially in Orthoptera. To attempt to mediate for potential problems with numts, we applied several recommendations by Song et al. (2008) including BLASTing sequences (NCBI), examining translated sequences for in-frame stop codons (for COII), and including multiple markers. Final, included sequences did not exhibit evidence of numts.

Morphology and non-molecular characters

A matrix of morphological and behavioral characters was developed based on features used historically to delimit groups. These characters have not been tested in any prior cladistic analysis, especially not with respect to S. isleta. The characters used in this analysis were those used for descriptions in Gurney’s (1940) paper describing S. isleta and by Otte (1984) as important characters for distinguishing taxa in the Chortophaga and Arphia genus groups. All characters were reexamined for all included taxa. We added a behavioral character, crepitation (courtship flight display sounds) that we believe is important to understanding relationships among band-winged grasshoppers, following the defined terminology of Weissman and Rentz (1980). We also added a life-history character, seasonality, because of its use in Otte’s (1984) classification. In total, 14 morphological characters, one behavioral and one life-history character were examined and coded for analysis (Table 2). Behavioral and seasonality character
Table 2. Morphological and other non-molecular characters and states used in analysis

<table>
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<td>12345678 90123456</td>
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- *Trimerotropis pallidipennis*
- *Arphia conspersa*
- *A. conspersa*
- *A. nietana*
- *A. pseudonietana*
- *A. simplex*
- *Chimarocephala elongata*
- *Chortophaga viridifasciata*
- *Encoptolophus fuliginosus*
- *E. otomitus*
- *E. subgracilis*
- *E. subgracilis*
- *Lactista aztecus*
- *L. elota*
- *L. gibbosus*
- *L. punctatus*
- *Leprus wheeleri*
- *Leuronotina orizabae*
- *Shotwellia isleta*
- *Tonomotus ferruginosus*
- *T. ferruginosus*
- *T. ferruginosus*
- *T. mexicanus*
- *Xanthippus corallipes*
- *X. montanus*
- *X. olancha*

All characters were treated as non-additive. Partial polymorphism indicated by $ (=states 1 and 2).

observations were made by D.C.L. A single character (character 6) is coded as polymorphic for *Arphia nietana*. Characters 1–5 are from Gurney’s (1940) description of *S. isleta*. Characters 6–14 are from Otte (1984). The analysis includes the following characters and states:

1. Lateral carinae of pronotum: distinct (0); not distinct (1).
2. Roundedness of posterior margin of pronotal disk: rounded (0); angled (1).
3. Intercalary vein: not present (0); present (1).
4. Radiate field: not enlarged (0); enlarged (1).
5. Head elevation: vertex elevated above level of pronotal disk (0); not elevated above pronotal disk (1).
6. Hind tibiae color: yellow (0); orange or red (1); blue (2); black (3); ivory (4).
7. Median pronotal ridge: pronounced (0); not pronounced (1).
8. Hind wing bands: complete (0); not complete (1).
9. Fastigium: narrow and shallow (*Shotwellia isleta*) (0); wider and deeper (1); wider and shallow (2).
10. Forewing cross banding: without pattern (0); vertical banding (1); spotted (2).
11. Fastigium median carinula: absent (0); present (1).
12. Hind wing color: red, orange or yellow (0); clear (1); blue (2).
13. Medial surface of hind femora: entirely black (0); banded (1).
14. Medial surface of hind femur: with color extended from tibia (0); without color extension (1); medical surface of hind femur with pattern colored same as tibia (2).
15. Crepitation; absent (0); buzz (1); pulse buzz (2); crackle (3).
16. Maturity season: spring (0); summer (1).

Male genitalia are important as a source of characters in many Orthoptera, including Acrididae (e.g., Roberts 1941). This does not, however, seem to be the case in the subfamily Oedipodinae (Roberts 1941). Otte (1984) provides illustrations of genitalic structures of *Chortophaga* group taxa, and shows that the genitalia are of little use differentiating the members of the *Chortophaga* genus group. We surveyed genitalic morphology across the included taxa, but did not find variation in this character system suitable for coding for the analysis, and did not include genitalic characters.

**Analysis**

Alignment for these genes was unambiguous in the case of COII since this gene is not length variable in these taxa. Alignment of 16S and 12S was conducted using Muscle (Edgar 2004) and the default settings.

Combined molecular, morphological and behavioral data were analyzed using equal-weights parsimony with NONA (Goloboff 1995) as implemented by WinClada (Nixon 2002). The commands used in NONA were “hold 10000”, “hold/50”, “mult 50” and “max*”. All characters were treated as non-additive. Gaps were treated as missing data. Trees and characters were examined under different optimizations using WinClada (Nixon 2002).

Branch support was evaluated using bootstrap values in NONA (Goloboff 1995) as implemented by WinClada based on 1000 replications sampling about 10% of characters, and saving the consensus for each replication. Partitioned and total Bremer support values (Baker and DeSalle 1997) were calculated in PAUP* (Swofford 2000) using a batch file generated by TreeRot (Sorensen 1999).

**Results**

The combined analysis resulted in three trees (length=1066, 919 with only informative characters mapped, CI=56, RI=71). One of those trees is shown in Fig. 5, and the consensus is shown in Fig. 6. The only conflicts between topologies were among outgroup taxa. In group taxa (the *Chortophaga* group) have the same topology in all three trees (Fig. 6). The *Chortophaga* group is monophyletic with high support (Fig. 6, bs=99, Bremer >15) and *S. isleta* is sister to the rest of the *Chortophaga* group, also with high support (Fig. 6, bs=100, Bremer >15). Additionally, our analysis revealed *Encoptolophus* (Scudder) to be paraphyletic with *Chortophaga* and *Chimarcepha* nested within it. *Arphia* is resolved as monophyletic with high support (Figs 5 and 6).
Discussion

Phylogenetics of the Chortophaga group of genera

Our analysis provides convincing evidence for the position of *Shotwellia isleta* within the *Chortophaga* genus group and supports the historical classification of *Shotwellia* as a monotypic genus sister to other genera in the *Chortophaga* genus group (Figs 5 and 6). Most of the branches, especially those subtending the *Chortophaga* group, are well supported (Figs 5 and 6). Our findings are consistent with Fries et al. (2007), who used mitochondrial markers COII, COI, cytb and nd5, and found that the *Chortophaga* group is a very old monophyletic clade of North American band-winged grasshoppers, distinct from other world Oedipodinae, and distinct from the other more recently evolved monophyletic clade of all other North American Oedipodinae.

Our results differ from Otte’s (1984) phylogeny in several important respects including the nesting of *Chortophaga* and *Chimarocephala* within *Encoptolophus*. Our
analysis confirms Otte’s (1984) assertion regarding the unique placement of *Shotwellia* with respect to other taxa in the group, albeit in a different phylogenetic position (Figs 5 and 6). Although we find convincing evidence for paraphyly of *Encoptolophus*, any change to the classification of these groups is beyond the scope of this project since our sampling of *Encoptolophus* did not include a substantial number of taxa (including type species). A more thorough revision (currently in progress) will be required to adequately place each species into appropriate groups and establish an improved classification.

We believe that molecular characters are particularly useful for the phylogenetic analysis of band-winged grasshoppers where morphological characters are less apparent than in other grasshoppers (Otte 1984). For example, morphology of the genitalia is important for differentiation of taxa across many grasshopper (Acrididae) groups; however, Roberts (1941) found little difference in the morphology of genitalic structures among the Oedipodinae and related subfamilies. Behavioral traits such as crepitation flight displays and pre-contact leg movements (Otte 1984) appear to be important in the Oedipodinae for reproductive isolation, and may have contributed to the conservation of genitalic morphology across the group. Crepitation patterns
do appear to correspond to phylogeny to some extent, with particular types of crepitative characterizations evident in some groups of related taxa (Weissman and Rentz 1980). Variation in life-history patterns also appear to correspond to phylogeny to some extent. For example, *Arphia* and *Chimarocephala* are characterized by some species that overwinter as nymphs, while most other taxa overwinter as eggs. Such life-history patterns could result from phylogeny, convergence or other factors, but have not been studied thoroughly.

**Natural history, distribution and conservation status**

*Shotwellia isleta* is rare and known from few records and localities (Fig. 3). We have found *Shotwellia* only in small and isolated ephemeral freshwater (non-saline) wetland environments of ephemeral lake beds within otherwise semi-arid Chihuahuan Desert landscapes. *Shotwellia* occurs on heavy clay soils that support plant communities that require frequent flooding, including the grass *Panicum obtusum* (Kunth), and forbs such as *Ratibida tagetes* (James) Barnhart. Otte (1984) reports *Shotwellia* from a “salt pan” in Zacatacas, Mexico, but we have not found *Shotwellia* associated with numerous saline ephemeral desert lake beds that we have surveyed across the Chihuahuan Desert. All three locations where we have found *Shotwellia* were highly impacted by concentrations of domestic cattle. All localities also served as watering points for rangeland livestock, and all were trampled and the vegetation and soils heavily impacted by cattle. Although the grasshopper appears to be surviving this disturbance, it is not clear how this impact may be changing or may have changed the population structure of this species.

*Shotwellia* and all other members of the *Chortophaga* genus group are somewhat ecologically different from typical band-winged grasshoppers in that they prefer relatively mesic and grassy habitats (D.C.L. unpublished data). Most other North American band-wing grasshoppers which are distantly related from the *Chortophaga* group, prefer areas of open bare soil, sand, or rocky substrates (D.C.L. unpublished). *Shotwellia* and some species of *Encoptolophus* (e.g., *E. pallidus* and *E. robustus*) are further unique in that they occur in isolated, or “island” patches of mesic and grassy habitats within widespread xeric semi-arid environments (Lightfoot, unpublished). The isolation of these taxa in such island habitats is likely recent in evolutionary time, following the development of North American desert and semi-arid landscapes since the end of the Pleistocene, approximately 10,000 years ago (Axelrod 1979, Betancourt et al. 1990), even though lineage of the *Chortophaga* group dates back approximately 40 million years (Fries et al. 2007). The implications for such a patchy distribution of subpopulations of the overall metapopulation, are that gene flow among subpopulations is probably restricted, and local extinction of subpopulations will reduce the overall genetic diversity of *Shotwellia* (Reed and Bryant 2000; Reed 2005). Given that the isolated island habitats that *Shotwellia* occurs in are heavily impacted by humans (i.e., domestic livestock) increases the probability of local subpopulation extinctions, and perhaps an overall decline in the fitness of the *Shotwellia* metapopulation. Research on the geographic distribution patterns and genetics of *Shotwellia* subpopulations are clearly needed in order to understand how imperiled the taxon is.
As a rare and localized species, *S. isleta* is considered to be globally imperiled (Nature Serve 2009), yet very little is known about the biology of the species, and because of discrepancies in past classifications, its phylogenetic status and uniqueness as a monotypic genus have also been questionable. We conclude that *Shotwellia* is indeed in a unique evolutionary position representing an earlier branching lineage relative to other related genera and species in the *Chortophaga* group (Figs 1 and 2). As such, *Shotwellia isleta* does indeed deserve special conservation status given that the species has a wide geographic distribution, but occurs rarely as subpopulations only in localized habitats that are highly impacted by human and livestock activities, and has a unique character combination.

**Distribution records for Shotwellia isleta** (Fig. 3)

USA, New Mexico, Bernalillo County, near Isleta Reservation, (type locality Gurney, 1940; Otte 1984). Specific collection location unknown.

USA, New Mexico, Cibola County, El Malpaís National Monument, near Grants (unpublished record), 34˚ 42’ 18” N 107˚ 59’ 27” W.

USA, New Mexico, Isaac’s Lake, Doña Ana County, near Las Cruces (unpublished record). 32˚ 27’ 35” N 106˚ 43’ 14” W.

Mexico, Durango, Gómez Palacio (Otte 1984). Specific collection location unknown.

Mexico, Zacatecas, 60 km northeast of Zacatecas City (Otte 1984).

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**References**


